

Studies of fenflumizole in man

with some aspects on platelets as a model tissue
for effects of nonsteroidal anti-inflammatory drugs

Ellen Vinge



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University Hospital of Lund, Lund, Sweden

STUDIES OF FENFLUMIZOLE IN MAN,
with some aspects on platelets as a model tissue
for effects of nonsteroidal anti-inflammatory drugs

By

Ellen Vinge
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AKADEMISK AVHANDLING

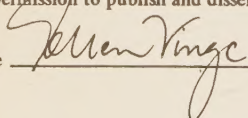
som för avläggande av doktorsexamen i medicinsk vetenskap
vid Lunds Universitet kommer att offentligen försvaras i föreläsningssal 2,
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Title and subtitle Studies of fenflumizole in man, with some aspects on platelets as a model tissue for effects of nonsteroidal anti-inflammatory drugs			
Abstract Fenflumizole, a non-acidic anti-inflammatory agent, was given to healthy volunteers as single oral doses of 0.1 mg/kg and as single intravenous doses of 0.1 mg/kg of cold and ¹⁴C-labelled compound. Bioavailability of oral doses was 49 %. After i.v. doses, the terminal half-life was about 120 hours. Plasma clearance was 0.55 liters/minute. Labelled compound was more rapidly eliminated from plasma than from blood, suggesting drug binding to blood cells. The volume of distribution at steady-state was about 3000 liters. Demethylated and conjugated metabolites were identified. Renal excretion of ¹⁴C accounted for 3.8 % of the total i.v. dose and about 50 % was recovered in the faeces over a one-week-period. Enterohepatic circulation, as well as extensive tissue binding of the drug, is suggested. In a concentration-dependent manner, fenflumizole suppressed the capacity of endogenous formation of thromboxane B₂ in blood and prolonged the lag phase in the platelet aggregatory response to exogenous arachidonic acid. These phenomena appeared to be parallel. Other effects on platelet aggregation were more complex. In order to obtain reference data of other anti-inflammatory drugs, similar ex vivo studies were conducted after single oral doses of indomethacin, indoprofen and lonazolac. All three compounds inhibited thromboxane generation reversibly and in a concentration-dependent manner with small differences in potency but all less potent than fenflumizole. However, the drugs diverged in their influence on the aggregatory response. This may reflect differences in the mechanisms of action within this group of agents.			
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We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot

The present thesis is based on the following articles, which will be referred to in the text by their Roman numerals (I - VI):

- I Midskov C, Vinge E & Andersson K-E:
On the pharmacokinetics of fenflumizole, a novel anti-inflammatory agent, after single oral administration to healthy subjects.
Acta Pharmacol. et Toxicol. 1984, 54:408-413
- II Vinge E, Arnold E, Rasmussen SN & Midskov C:
Single intravenous and oral doses of fenflumizole: pharmacokinetics and effects on prostanoid formation.
Acta Pharmacol. et Toxicol. 1985, 57:121-129
- III Vinge E, Midskov C & Arnold E:
Pharmacokinetics of ^{14}C -fenflumizole after intravenous administration to man.
Submitted for publication.
- IV Vinge E, Corell T & Andersson K-E:
Effects of fenflumizole on aggregation ex vivo of human platelets and formation of thromboxane B_2 and 6-keto-prostaglandin $\text{F}_{1\alpha}$.
Eur. J. Clin. Pharmacol. 1984, 26:711-717
- V Vinge E:
Arachidonic acid-induced platelet aggregation and prostanoid formation in whole blood in relation to plasma concentration of indomethacin.
Eur. J. Clin. Pharmacol. 1985, 28:163-169
- VI Vinge E & Björkman S:
Compared effects of the two antiinflammatory drugs indoprofen and lonazolac on thromboxane generation and on platelet aggregation, related to plasma concentrations after single oral doses.
Submitted for publication.

CONTENTS

INTRODUCTION	1
Nonsteroidal anti-inflammatory drugs	2
Platelets	6
Platelet structure	7
Aims	9
METHODS	10
Subjects	10
Procedures	10
Drug concentration in plasma	10
^{14}C-radioactivity	12
Prostanoid generation	12
Platelet aggregation	12
Pharmacokinetic analysis	15
RESULTS	16
Pharmacokinetics of fenflumizole	16
Pharmacokinetics of indomethacin, indoprofen and lonazolac	17
Prostanoid formation	17
Thromboxane B_2	17
6-keto-PGF $_{1\alpha}$	21
Platelet aggregation	21
DISCUSSION	24
Kinetics	24
Fenflumizole	24
Indomethacin, indoprofen, lonazolac	26
Prostanoid biosynthesis	27
Levels of TXB $_2$ -immunoreactive substance	27
Levels of 6-keto-PGF $_{1\alpha}$ -immunoreactive substance	32
Platelet aggregation	33
Platelet aggregation induced by AA	33
Platelet aggregation studies following drug administration	34
Concentration-effect relationships	35
Platelets in disease	40

SUMMARY AND CONCLUSIONS	43
ACKNOWLEDGEMENTS	45
REFERENCES	46
APPENDIX (papers I-VI)	

ABBREVIATIONS USED IN THE TEXT:

AA = arachidonic acid

AA_{1 min} = concentration of AA that gives a 1 minute lag phase in the aggregatory response

ADP, ATP = adenosine-di-phosphate, adenosine-tri-phosphate

AMI = acute myocardial infarction

ASA = acetyl salicylic acid

AUC = area under the curve

CO = cyclooxygenase

ECG = electrocardiogram

HETE = hydroxy-tetraenoic acid

HPETE = hydroperoxy-tetraenoic acid

HPLC = high performance liquid chromatography

IC₅₀ (IC₉₀) = the concentration of a compound at which a studied activity is inhibited by 50 % (90 %)

LT = light transmission

LT_{50%} = 50 % of the maximum increase in light transmission

LTA₄ - LTD₄ = leukotriene A₄ - leukotriene D₄

LO = lipoxygenase

LX-A, LX-B = lipoxin A, lipoxin B

M = molar = mols per liter

NSAID = non steroid anti-inflammatory drug

PG (PGE₂, PGF_{1α} etc.) = prostaglandin (prostaglandin E₂, prostaglandin F_{1α} etc.

PGI₂ = prostaglandin I₂ = prostacyclin

PRP, PPP = platelet rich plasma, platelet poor plasma

RIA = radioimmunoassay

SD = standard deviation

SEM = standard error of the mean

t_{1/2} = half-life

TIA = transitory ischemic attack

UV = ultraviolet

INTRODUCTION

For every drug it is important to know not only its effect on the body, but also the effects of the body on the drug (Levy, 1984). Knowledge of the kinetics of drug disposition will help in understanding the kinetics of the effects, beneficial as well as adverse. However, measurable concentrations of a drug in body fluids do not necessarily mean that a pharmacological effect is exerted on the body and failure to measure drug concentrations does not exclude drug actions. The kinetics of the chemical compound do not always parallel the kinetics of the effects. It is therefore desirable to study pharmacokinetics and drug actions concomitantly.

The pharmacokinetics of drugs are greatly dependent on their chemico-physical properties, such as molecular weight, solubility in fat and water, electrical charge and existence of chemical groups that have affinity for certain enzymes or receptors. These properties will also influence the pharmacological activity.

If there is a correlation between drug effects and concentrations in vivo, the pharmacokinetic data can be used for calculations of adequate dosage regimens. The effects may not always be determined by the concentrations in plasma but in other tissues, more or less easily accessible to the drug. Evidence for this can sometimes be revealed indirectly by careful analysis of the kinetics of the concentrations in plasma. However, concentrations of the drug may appear dissociated from drug effects in cases where metabolites with prominent pharmacological activity are also formed, in cases where a biochemical system is saturated by low concentrations of the drug, in cases where the effects are delayed or in cases where drug effects are irreversible (e.g. destruction of proteins that are not replaced immediately).

In the present study, the relationship between plasma-concentrations and drug effects of some agents with anti-inflammatory activity were studied, with the use of platelets as a model

tissue. These agents have in common that they are chemically not related to the anti-inflammatory steroids formed in the adrenal cortex and are, therefore, commonly referred to as non steroidal. The emphasis was on fenflumizole, a novel compound which had not previously been studied in humans, but for comparative evaluation of the activity, three other non steroidal anti-inflammatory drugs were studied in a similar way.

Non steroidal anti-inflammatory drugs (NSAIDs)

It has been known since 1827 that a glycoside extracted from the bark of the willow tree (*Salix alba*) and called salicin, and derivatives of this compound, were active against agues and fever (Flower et al., 1985). Since 1876, sodium salicylate has been used in the treatment of rheumatic disorder (Hedenius, 1916). Acetylsalicylic acid (ASA, Aspirin) was synthesized in 1899 (Flower et al., 1985) and is still widely used in clinical practice. Empirically, salicylates were known to have important pharmacological actions in several tissues, although the mechanism of action was not clear. In 1971, acetylsalicylic acid was shown to be an inhibitor of the enzyme cyclooxygenase which transforms arachidonic acid (AA) into prostaglandins and related compounds (Vane, 1971; Smith & Willis, 1971; Ferreira et al., 1971). This property was shared by indomethacin, and to a lesser extent, by salicylic acid, a structurally different drug which was also established as an anti-inflammatory agent. Prostaglandins had been the subject of great interest during the decade preceding this discovery, which made it possible to intensify the research in this field even more during the years to follow. Some cyclooxygenase products were discovered that were not strictly prostaglandins, the potent aggregatory substance thromboxane A_2 (TXA₂) produced by platelets (Hamberg et al., 1975) and the potent antiaggregatory prostacyclin (PGI₂) produced by endothelial cells (Moncada et al., 1976). Continued research led to the discovery of the 5-, 12, and 15-lipoxygenase pathways, which transform AA into hydroperoxy- and hydroxytetraenoic acids (HPETE, HETE) and leukotrienes A₄, B₄, C₄ and D₄ (LTA₄-LTD₄). Recently, the lipoxins A and B (LX-A, LX-B),

have been synthesized from 15-HPETE (Serhan et al., 1984). AA-metabolism is presented schematically in Figure 1.

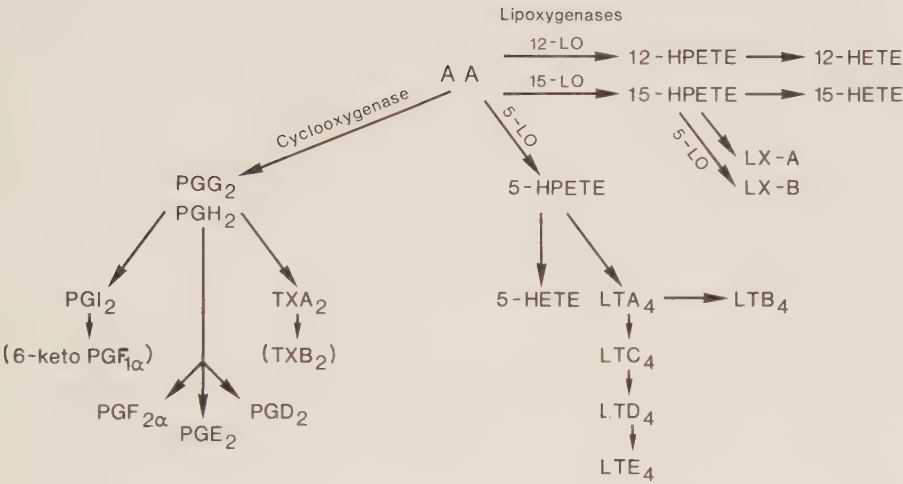


Figure 1.
Simplified scheme of arachidonic acid metabolism.

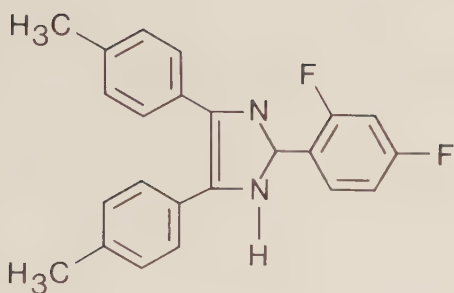
Some of the prostaglandins known around 1970 had been connected with inflammatory lesions. Inhibition of the cyclooxygenase pathway of arachidonic acid metabolism appeared to be a common property for drugs with "aspirin-like" actions and this mechanism could account for some of their therapeutic effects (Vane, 1971). This conclusion facilitated the search for new drugs with similar beneficial actions and would, hopefully, help in reducing the number of side effects of such compounds.

Anti-inflammatory drugs are usually lipophilic and weakly acidic, but in other aspects they are different from each other. They can be divided into groups according to their chemical structures. The following classification was proposed by Verbeeck et al. (1983):

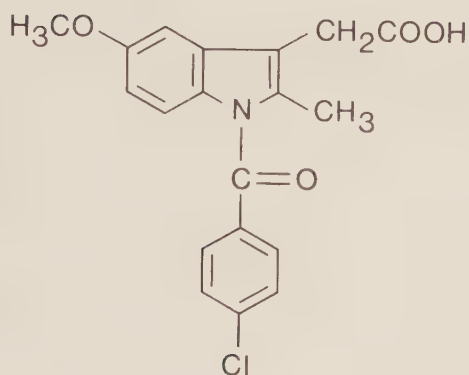
1. salicylic acid derivatives
2. pyrazoles
3. indole derivatives
4. propionic acid derivatives
5. phenylacetic acid derivatives
6. oxicams

Fenflumizole (2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole (Figure 2) is a non-acidic substance belonging to the series of substituted triaryl-imidazoles described by Lombardino and Wiseman (1974), and had been shown to be a potent inhibitor of prostanoid formation in vitro and in vivo in laboratory rodents (Corell et al., 1983). It was found to have potent analgesic, antiinflammatory and antipyretic effects in animal models (Corell & Hasselmann, 1983), but a low ulcerogenicity and a low acute toxicity (Corell & Hasselmann, 1983).

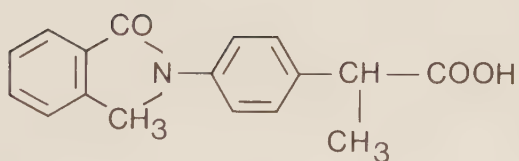
Indomethacin is an indole derivative (Figure 2) which has been in clinical use as an anti-inflammatory drug for many years. It is often used as a reference compound for inhibition of prostaglandin synthesis and for its anti-inflammatory effects.



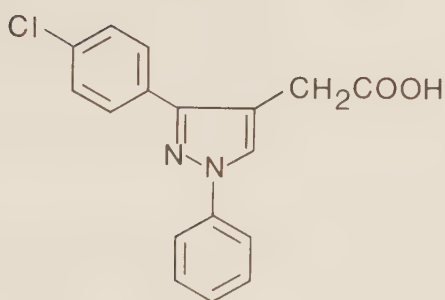
Fenflumizole



Indomethacin



Indoprofen



Lonazolac

Figure 2. Structural formulas of fenflumizole, indomethacin, indoprofen and lonazolac.

Indoprofen is a propionic acid derivative (Figure 2) chemically related to ibuprofen, naproxen and ketoprofen (Ceserani et al. 1979). Lonazolac is a pyrazole acetic acid derivative (Figure 2) like phenylbutazone and sulphinpyrazone (Rainer et al., 1981), with potent pharmacological effects in animal models as described by Riedel (1981). These two drugs had been under clinical investigation for anti-inflammatory and analgesic effects for some years when the present study was initiated.

Platelets

The conclusion by Vane et al. in 1971 regarding the mechanism of action of aspirin was based on experiments using various tissues, among them platelets. It had been observed in the 19th century that salicylates could cause hemorrhage and purpura which could not be attributed to a local effect. (Pullman, 1889; Binz, 1893). Profuse bleedings in connexion with ASA-treatment were an early finding (Fischer, 1903). This could in part be explained by decreased levels of vitamin K-dependent coagulation factors but there were also additional effects on hemostasis (Meyer & Howard, 1943; Smith & MacKinnon, 1951). It was found that aspirin reduces platelet adhesiveness to collagen (O'Brien 1968) and inhibits the second wave of aggregation induced by ADP (Zucker & Peterson, 1968) and adrenaline (O'Brien 1968). Smith and Willis demonstrated in 1971 that ASA and indomethacin given to volunteers inhibited production of prostaglandins by platelets in response to high concentrations of thrombin, but not the platelet release reaction. They also reported that sodium-salicylate was about 20 times less potent than acetylsalicylic acid.

Platelet aggregation (Born, 1962; O'Brien, 1962) is now regularly used as an in vitro screening test for studies of prostaglandin-inhibiting effect of various substances. Several compounds are known to induce platelet aggregation, and PG-formation from endogenous AA is seen as a secondary response to many of them, e.g. adenosine-5'-diphosphate (ADP), collagen and adrenaline (Siess et

al., 1981). Exogenous AA can also induce platelet aggregation (Vargaftig & Zirinis, 1973), which is believed to occur independently of release of proaggregatory substances from platelet granules (Kinlough-Rathbone et al., 1976; Burch & Lamb, 1983) but as a response to endoperoxides and thromboxane formed via the cyclooxygenase pathway (Hamberg et al., 1974, 1975). However, platelet function can be altered by many other agents that do not interfere with prostaglandin generation and the inhibitory effects measured in aggregation tests depend not only on the concentration of the drug studied, but also on the mechanism of action of the aggregation substance used and on its concentration (De Caterina et al., 1985).

Platelet structure

In the resting state, the platelet is discoid with a diameter of about 2-3 μm . The shape is maintained by a peripheral ring of contractile microtubuli (Figure 3). Platelets are formed from the megakaryocytes and have, themselves, no cell nucleus, but contain cellular organelles. Characteristic for the platelet are the dense bodies, which contain high concentrations of serotonin, ATP, ADP and Ca^{++} , and the alpha-granules which contain several proteins, some of which are specific for platelets (e.g. beta-thromboglobulin, platelet factor 4, platelet derived growth factor). There are also lysosomes, mitochondria and glycogen granules in the cytoplasm. The surface of the platelet is increased by invaginations of the cell membrane which form canaliculi in the platelets and are, in turn, connected to the dense tubular system, which corresponds to the sarcoplasmic reticulum in muscle cells (see Gordon, 1981).

The platelets have properties in common both with smooth muscle cells (contractility) and with neurons (loaded granulae which are released upon stimulation of the cell) and have often been used as cell models for various purposes.

When platelets are activated, they change their shape and become spherical and form pseudopodia, i.e. extensions of the

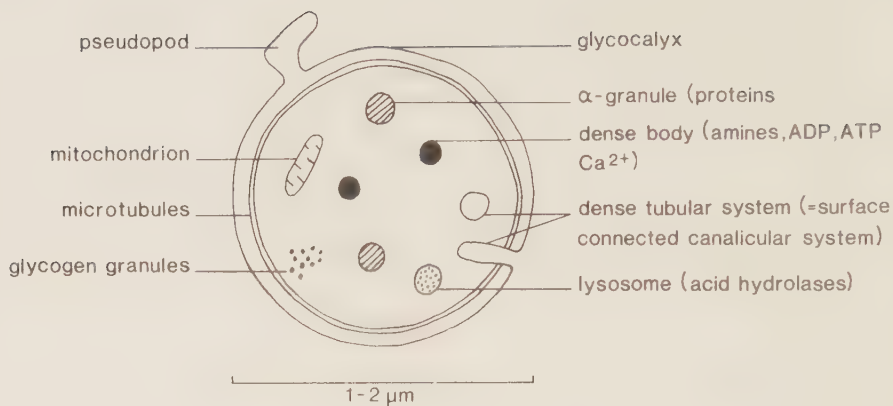


Figure 3. Platelet morphology.

cytoplasm which facilitate contacts with other cells. Bridges between platelets are formed and aggregates appear. The aggregation may be reversible or irreversible. Aggregation is usually accompanied by secretion of granule contents and of generation of thromboxane, two events which promote a positive feedback of the aggregation process. Even though these functions interact, they can also occur separately, as demonstrated by Smith and Willis in 1971. Several pathways of biochemical reactions promoting aggregation in response to different stimuli appear to exist (Vargaftig et al., 1981).

Platelet aggregation is thus a complex response with several processes involved. The reaction can be useful for the study of cell physiology, but can also be employed for the study of the

pharmacodynamics of drugs. In the latter case, it is usually desirable to involve as few biochemical events as possible. For the study of drugs with supposed effect on the cyclooxygenase enzyme, it therefore seems appropriate to stimulate platelets with AA instead of with other agents that may possibly be more physiologic in hemostasis, such as ADP, thrombin or collagen. However, since aggregation in response to AA can still be considered as the sum of a cascade of events, it is also desirable to measure the metabolite or metabolites formed in the absence and in the presence of the drug under study and to relate these results to the aggregatory response. In platelets, the dominating product of the cyclooxygenase pathway of AA metabolism is thromboxane A₂ (TXA₂) (Hamberg et al., 1975) which is quickly inactivated to its stable metabolite thromboxane B₂ (TXB₂). This substance is commonly used as a marker of cyclooxygenase activity in platelets.

Aims

The aims of the present study have been

- to describe the profile of concentrations of fenflumizole in plasma after single oral and venous doses to healthy subjects
- to estimate the degree of bioavailability of oral doses of fenflumizole
- to investigate how fenflumizole is eliminated from the body
- to study in vivo effects of fenflumizole in healthy humans, using platelets as a model tissue and to relate the effects on platelet aggregation induced by arachidonic acid and on endogenous TXB₂-generation to the concentrations of the drug
- to evaluate the effects on platelet aggregation and on TXB₂-formation of some other drugs with similar actions but different chemical structure (indomethacin, indoprofen, lonazolac) and to relate the effects to the concentrations in plasma of each of these drugs.

METHODS

Subjects

A total of 16 volunteers were enrolled in the present studies. A physical examination was conducted prior to enrollment and ECG and laboratory tests of hematology indices, hepatic and renal functions were normal in all subjects. Data on the subjects and in which studies they took part are presented in Table 1.

Procedures

The drugs were given in single oral or intravenous doses. Fenflumizole was given orally as a suspension in doses of 0.1, 0.5, 1.0 and 2.0 mg/kg, and intravenously as an infusion (0.1 mg/kg) over a 5 minutes period (I, II, III, IV). Indomethacin was given orally as a suspension in the dose 1 mg/kg (V). Indoprofen was given in capsules of 100 mg and lonazolac was given as tablets of lonazolac-Ca 200 mg (VI). At various times after the drug administration, blood samples were taken for analyses of drug concentration in plasma (I-VI), of radioactivity in blood and plasma (III), for measurement of prostanoid forming capacity (II, III, IV, V, VI) and for tests of platelet aggregation (IV, V, VI). Urine was collected at intervals after the dose administration for 24 hours (I) and for 168 hours (III). Faeces were collected for 168 hours following a dose of ^{14}C -fenflumizole (IV).

Drug concentration in plasma

a/ Fenflumizole was measured by high performance liquid chromatography (HPLC) using UV- or fluorescence detection as described by Midskov (1983).

b/ Indomethacin was determined by gas-chromatography as described by Möller-Jensen (1978).

c/ Indoprofen was determined by HPLC according to Wåhlin-Boll et al. (1981), with a slight modification of the procedure (VI).

Table 1

Data on subjects

Subj	Year of birth	Sex	Paper					
			I	II	III	IV	V	VI
A	-49	M	x	x	x	x	x	x
B	-57	M	x	x		x	x	
C	-50	M	x	x	x	x		
D	-56	M	x	x		x	x	x
E	-58	M	x	x	x	x	x	x
F	-40	M	x	x		x		
G	-46	M	x	x		x	x	
H	-56	M	x	x		x	x	
I	-37	F					x	x
J	-41	F					x	x
K	-53	F					x	
L	-50	F					x	x
M	-53	F					x	x
N	-40	F					x	x
O	-51	M			x			
P	-55	M			x			

d/ Lonazolac was analysed by HPLC applying UV- and fluorescence detection as described by Huber and Zech (1982).

¹⁴C-radioactivity

Radioactivity in plasma and urine was measured in crude samples and in blood and faeces following total combustion.

Prostanoid generation

Prostanoid generation was measured in blood which was allowed to clot at 37°C for one hour, with a slight modification of the method described by Patrono et al. (1980). Serum was analyzed for its content of thromboxane B₂- and 6-keto-prostaglandin F_{1α}-immunoreactive substance by radioimmunoassay (RIA), with assay kits purchased from New England Nuclear (Boston, Mass., USA). Standard curves were derived with the same proportions of assay buffer and control plasma as used for unknown samples of serum. All tests were made in duplicates. For details of the procedure see (V).

Platelet aggregation

Platelet aggregation was carried out essentially as described by Born (1962) and O'Brien (1962). The equipment was a HU-aggregometer connected to a Vitatron recorder (Figure 4). Platelet rich plasma (PRP) was prepared from blood anticoagulated with sodium-citrate 0.129 M. Aggregation was induced by various concentrations of AA which had been dissolved in ethanol and sodium carbonate. The aggregation response was evaluated in different ways from the recorder tracings (Figure 5):

- A. The lag phase to the end of platelet shape change and to the start of the increase in light transmission (IV, V, VI).
- B. The absence or presence of irreversible aggregation (V, VI).
- C. The rate of aggregation, which was measured as the maximum slope of the curve (VI).

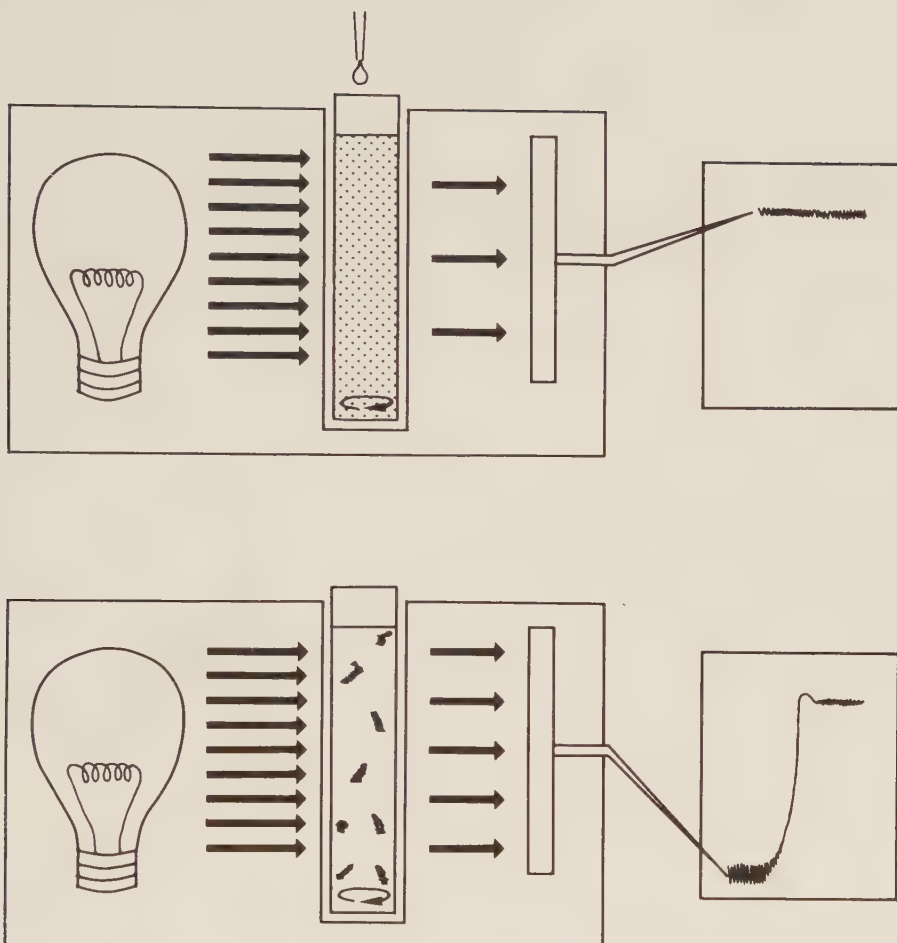


Figure 4. Principle for platelet aggregation according to Born. Platelets are suspended in plasma, or buffer, and stirred with a small magnetic rod. Light transmitted through this suspension is partly scattered. When an active agent is added to the suspension, aggregates of platelets are formed, and light transmission increases.

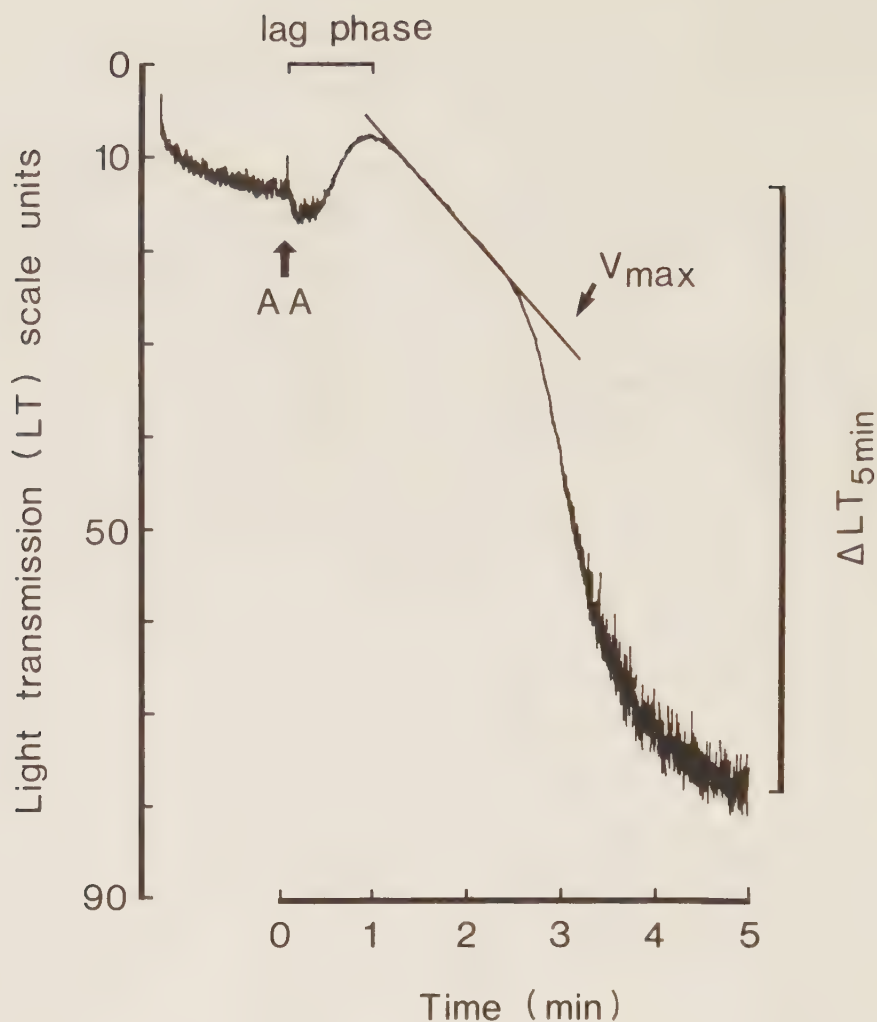


Figure 5. Typical tracing of platelet aggregation in plasma induced by arachidonic acid. Inductive agent added at arrow. The lag phase, the slope of the curve (= rate of aggregation) and the change in light transmission (LT) from 0 to 5 minutes are demonstrated. When reversible aggregation occurs, the tracing shows an initial increase in light transmission, but then returns towards the base-line (figure from VI).

D. The extent of aggregation which was measured as the change in light transmission (LT) between base-line and a point after a certain time interval (5 minutes) (VI).

Pharmacokinetic analysis

Linear analysis was applied to describe the curves of drug concentrations in plasma versus time. Formulas applied for calculation of kinetic parameters are presented in the methods section of the respective paper. Computer assistance for calculations was used in papers II and III.

RESULTS

Pharmacokinetics of fenflumizole

Kinetics of fenflumizole were independent of dose in the dose-range studied (0.1-2 mg/kg), as seen from the linear relation between areas under the concentration-time curve over 24 hours (AUC_{0-24}) and the oral doses (II). Linear analysis of the kinetics in plasma was therefore applied.

The pharmacokinetics of orally administered fenflumizole could be described by a two compartment model. The drug was quickly absorbed and maximum concentrations were found at 0.5-1 hour after drug administration. The two phases of elimination from plasma had half-lives ($t_{1/2}$) of about 1 hour and 15 hours, respectively.

After the intravenous dose of unlabelled fenflumizole (II) the kinetics could be described by a three compartment model, with one very fast phase immediately after the end of the short infusion, a second phase with a half-life of about 1 hour and a final phase with a half-life of approximately 15 hours. After the intravenous dose of labelled compound (III), blood sampling was extended over one week, which revealed a slow terminal elimination phase with a median half-life of 119 hours. The concentration-time curve over 168 hours could be described by a four-compartment model.

Bioavailability of orally administered fenflumizole was calculated to 0.49. Clearance of the drug from plasma was 0.55 l/min.

The volume of distribution at steady-state was calculated from the four-compartment model (III) to 1 260 - 3 800 liters. Urinary excretion of fenflumizole in 24 hours following an oral dose was <10/100. Following a single intravenous dose of labelled drug, 2.4 - 3.3% of the radioactivity was recovered in the urine within the first 36 hours and 3.1 - 4.5% after 168 hours.

The faecal excretion of fenflumizole following an intravenous dose of ^{14}C -fenflumizole was 47 - 67% over a one week period.

Pharmacokinetics of indomethacin, indoprofen and lonazolac

The pharmacokinetics after oral doses of these drugs were not analysed in detail. The maximum plasma concentrations were 8-10 $\mu\text{g/ml}$ for indomethacin, 14-16 $\mu\text{g/ml}$ for indoprofen and 2-3 $\mu\text{g/ml}$ for lonazolac. All appeared to have two phases in the decline of plasma concentrations. The half-life of the terminal phase was 4-11 hours for indomethacin, 4-8 hours for indoprofen and 4-6 hours for lonazolac. Clearance of indomethacin was calculated assuming bioavailability of 100 % (Alvan et al., 1975) to 0.043-0.094 l/kg/h .

Prostanoid formation

Standard curves of the radioimmunoassays performed in our laboratory are shown in Figure 6. (Means $\pm$ SD of standards)

Thromboxane B_2

Mean concentrations of thromboxane B_2 measured by radioimmunoassay in drug-free serum prior to drug administration ranged from 180 to 270 ng/ml (Table 2). TXB_2 -levels measured in samples taken after drug administration were calculated as % of the pre-dose value for the same individual. In almost all cases, >95% inhibition was found when the highest drug-concentrations were measured. The capacity of thromboxane generation was gradually recovered as drug-concentrations decreased. For each drug, a concentration-dependent relationship between TXB_2 - and drug-levels was found. The total concentration of the drug in plasma at which the TXB_2 generative capacity was inhibited by 50% (IC_{50}) was estimated as

18-20 ng/ml = $4.6\text{-}5.1 \times 10^{-8}$ M for fenflumizole (II, IV)

100 ng/ml = 2.7×10^{-7} M for indomethacin (V),

40 ng/ml = 1.5×10^{-7} M for indoprofen (VI),

69-91 ng/ml = $2.2\text{-}2.9 \times 10^{-7}$ M for lonazolac (VI).

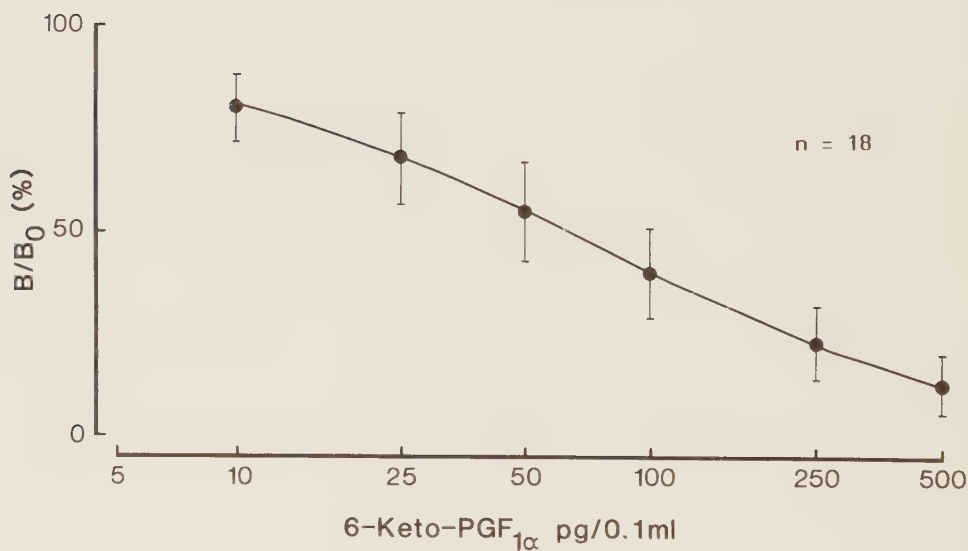
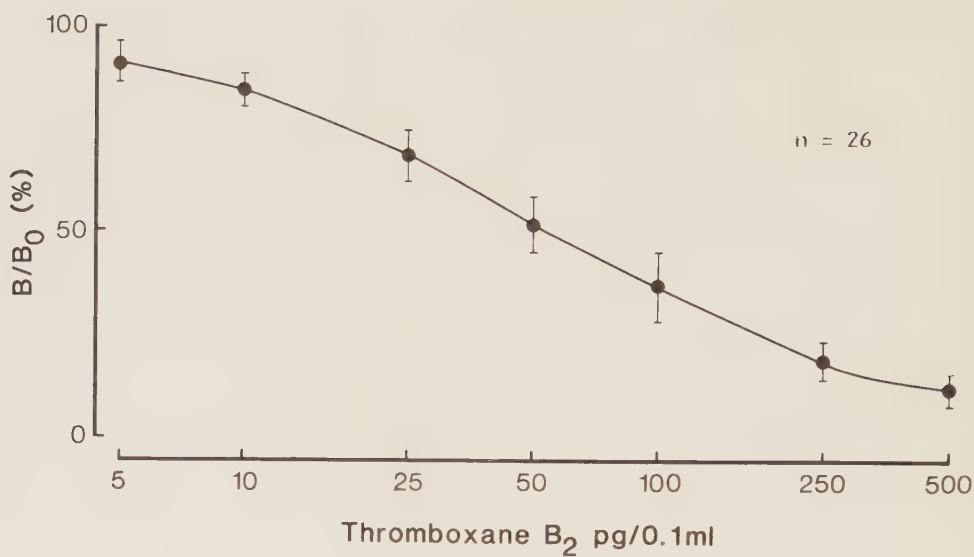


Figure 6. Standard curves for radioimmunoassays of thromboxane B_2 (upper) and of 6-keto-prostaglandin $F_{1\alpha}$ (lower). Means of standards $\pm$ SD.

Table 2

TXB₂-levels in serum (ng/ml)

	1983		1982	1983	1984	
Subj.	II		IV	V	VI	
	i.v	p.o			Indop.	Lonaz.
A	185	205	186	205	125	130
B	220	280	250	250		
C	210	188	230			
D	295	75	206	270	155	52
E	295	275	450	320	250	280
F	124	150	220			
G	295	425	445	380		
H	255	190	175	66		
I				225	220	210
J				240	250	195
K				190		
L				500	270	290
M				152	200	185
N				250	230	115
mean	235	217	270	254	212	182
±SEM	22	38	40	32	18	29

Table 3

6-keto-PGF_{1α}-levels in serum (ng/ml)

	1983		1982	1983
Subj.	II		IV	V
	i.v.	p.o		
A	0.26	0.31	0.88	0.31
B	0.42	0.42	1.15	0.58
C	0.39	0.40	0.91	
D	0.42	0.16	1.06	0.46
E	0.52	0.52	1.17	0.36
F	0.36	0.40	1.03	
G	0.44	0.54	1.18	1.25
H	0.64	0.46	0.85	<0.10x
I				0.83
J				0.60
K				0.92
L				0.72
M				0.55
N				0.56
mean	0.43	0.40	1.03	0.60
±SEM	0.04	0.04	0.05	0.09

x calculated as 0.10

The concentrations in plasma at which TXB₂ generation was inhibited by 90% (IC₉₀) was
about 0.1 µg/ml = 2.6×10^{-7} M for fenflumizole (II, IV)
about 0.5 µg/ml = 1.4×10^{-6} M for indomethacin (V)
about 0.3 µg/ml = 1×10^{-6} M for indoprofen (VI)
about 0.6 µg/ml = 2×10^{-6} M for lonazolac (VI).

6-keto-PGF_{1α}

Mean concentrations of 6-keto-PGF_{1α}-immunoreactive material in serum were 0.4 - 1 ng/ml (Table 3). The levels decreased after drug administration in a parallel mode to that of TXB₂. IC₅₀ was estimated as 40 and 53 ng/ml ($=1 \times 10^{-7}$ M and 1.3×10^{-7} M) for fenflumizole (IV), II) and, for indomethacin, as 0.100 µg/ml (2.7×10^{-7} M; V).

Platelet aggregation

Aggregation induced by AA was highly sensitive to the presence of any of the drugs. The tracings formed complex curves, in which different events or phases related to concentrations of AA and to concentrations of the respective drugs could be evaluated.

A. The duration of the lag phase was dependent on the concentration of AA and on the concentration of the drug. In (IV), a linear relationship was found between the AA_{1 min} (concentration of AA giving a lag phase of 1 minute) and the log concentration of fenflumizole in plasma. For each subject, an equation for the line was calculated. The linear correlation was significant for all but one subject.

For indomethacin and indoprofen, the AA_{1 min} could only be estimated less precisely, but a similar correlation to the log concentration of drug in plasma was suggested.

For lonazolac, as for fenflumizole, values for AA_{1 min} could be calculated from titration with various concentrations of AA. As with the other compounds, the AA_{1 min} appeared to be dependent on the drug concentration in plasma.

B. In drug free PRP, no reaction, or only a weak reversible aggregation, was usually seen when PRP was stimulated with less than 300-500 μM AA. The threshold value for aggregation varied between individuals. Complete and irreversible aggregation was usually elicited by AA 500-1500 μM , but with 1500-2000 μM or higher concentrations of AA reversible aggregation tended to occur. This was also reflected by the rate and extent of aggregation (see below).

In the presence of any of the drugs, the reaction of platelets was attenuated in a concentration-dependent manner. Irreversible aggregation could not be elicited when drug concentrations were at their highest, but as the drug disappeared from plasma, the reactivity returned. The threshold value for AA needed to elicit irreversible aggregation thus successively decreased as drug concentrations decreased, but the tendency to reversible response to AA 2000 μM or more was maintained.

C. The rate of aggregation was measured as the slope of the curve in the first phase of aggregation, i.e. immediately following the slope change. The rate was dependent on both the AA concentration in PRP and the presence of drug. As with the extent of aggregation, the response to AA first increased with higher concentrations, but then again declined. In the initial separate studies with fenflumizole and indomethacin, it was observed that the rate of aggregation was more inhibited by indomethacin than by fenflumizole (results not presented) and that the difference could not easily be explained by large differences in the suppressive effects on TXB_2 -formation. In (VI), a similar difference was found between indoprofen and lonazolac. Indoprofen behaved more like indomethacin and lonazolac more like fenflumizole.

D. The extent of aggregation was measured in VI. At low concentrations of AA or when a drug was present, the aggregatory response was weak and reversed after having reached not more than about 30% of the maximum possible LT. The tracing then returned towards the baseline. With higher concentrations of AA, a second phase followed in the tracing starting at about LT 30% and ended with the formation of large aggregates and irreversible aggregation. At

high concentrations of AA (2000 μ M or more) the second phase appeared, but the large aggregates again disaggregated and the tracing rapidly returned towards baseline. The start of the second phase appeared to be an all-or-none phenomenon.

DISCUSSION

Kinetics

Fenflumizole

Fenflumizole was quickly absorbed from the gastrointestinal tract. This indicates rapid penetration over biological membranes, which might be expected from its high lipophilicity (Oldendorf, 1974). The bioavailability was lower than for many other NSAIDs, but in the same order as for diclofenac and tolfenamic acid (Verbeeck et al., 1983). The high degree of extrarenal clearance, suggests that hepatic first pass elimination accounts for the amount of an administered oral dose that never reaches the systemic circulation. Furthermore, the estimated value for the hepatic extraction ratio suggests that bioavailability of fenflumizole can be liable to variations if liver blood flow is altered, or if it is ingested with other drugs that compete for the same metabolizing enzymes. It is also probable that ingestion with food may alter bioavailability.

Fenflumizole seems to be rapidly distributed to the tissues and the volume of distribution is high. For other NSAIDs, the volume of distribution is usually reported to be in the range of 0.1 - 2 l/kg (Verbeeck et al., 1983; Helleberg, 1981) whereas for fenflumizole, the volume of distribution at steady-state was calculated to around 3000 liters, corresponding to 38 l/kg (III). This is in the same order of magnitude as for desmethylinipramine, but still much less than for chloroquine (Rowland & Tozer, 1980). From animal studies, it is known that ^{14}C -fenflumizole is retained in the adipose tissue and the skin for a long time (Midskov & Arnold, pers. comm.), and the present study demonstrated binding to or uptake by blood cells. These tissues may function as reservoirs which readily take up fenflumizole, possibly bind it, and then release the drug slowly. If so, an extremely slow terminal elimination phase with low drug concentrations would be expected and was indeed found (III). After single doses, such a phase may not be recorded if the elimination rate is low and if sampling is

carried out over a relatively short period, especially if the concentrations fall close to or below the determination limit. As with fenflumizole, the terminal half-lives of indomethacin and indoprofen have also been found to be longer than initially reported when more extended blood sampling has been conducted (Alvan et al., 1975; Caruso et al., 1980; Lund et al., 1984)

The non-acidic nature of fenflumizole may account for the different pattern of distribution than that found for most NSAIDs. In general, weak acids reach lower concentrations in body fluids that have a pH lower than plasma, whereas weak bases tend to accumulate, since the ionized drug molecule does not readily pass biological membranes. In theory, this may affect the distribution of NSAIDs to inflammatory lesions in synovial tissue where pH is lowered (Wallis & Simkin, 1983) or to the ventricular mucosa, in which the cells have a high intracellular pH as compared with the surrounding extracellular fluid which has a very low pH (Garner, 1977). Indeed, salicylate seems to be accumulated in parietal cells of the ventricular mucosa (Brune et al., 1977). The absence of an acid group in fenflumizole may also result in a different pattern of binding to plasma proteins, which in turn may influence the apparent volume of distribution.

The clearance of fenflumizole from plasma was high and was almost entirely extra-renal. The renal excretion of unchanged drug was about 0.0001 % of the total dose during a one week period, and only about 4 % of labelled drug (including metabolites) was recovered in the urine in total. Low figures (< 1 %) have also been reported for renal excretion of e.g. unchanged phenylbutazone (Aarbakke et al., 1977), and diclofenac (Willis & Kendall, 1978), whereas about 7-15 % of an oral dose of indoprofen was excreted unchanged in the urine (Fuccella et al., 1973).

It has been proposed that indomethacin is partly eliminated by tubular secretion (Helleberg, 1981). With ¹⁴C-fenflumizole, a non-linear relationship between renal clearance and plasma concentration of radioactivity was found, which may indicate a tubular saturable reabsorption process (III). However, it may also

indicate changes in the fraction of the drug bound to plasma proteins, which has been found with naproxen (Runkel et al., 1974). The extremely low renal clearance of unchanged fenflumizole is compatible with a very high degree of protein binding, but may also support the possibility of renal reabsorption. For salicylate, both passive and carrier-mediated reabsorption seem to occur, as well as some degree of tubular secretion (see Möller & Sheikh, 1983).

The main part of fenflumizole was excreted in the bile or via the intestinal mucosa into the faeces. A high extent of biliary excretion corresponds well with an important first pass metabolism, which was also suggested from the estimation of the hepatic extraction ratio. The two identified demethylated metabolites were present in small amounts and disappeared rapidly from plasma. The results from administration of ^{14}C -fenflumizole suggest formation of additional metabolites which are present in plasma and excreted in the urine. They remain to be identified.

The kinetics of fenflumizole showed a low degree of intra-individual variability in the eight subjects who received several doses. However, an enterohepatic circulation of the drug cannot be ruled out, and this would result in greater variations in the kinetics when fenflumizole is given in repeated doses or to non-fasting individuals. Enterohepatic recycling would be in accordance with the slow terminal elimination phase, and it could explain the humps in the concentration time curves (III) and possibly the irregular plasma-concentration pattern found in one individual in the bioavailability study (II). These observations indicate that linear kinetics may provide poor models for fenflumizole disposition in man.

Indomethacin, Indoprofen, Lonazolac

The plasma concentrations and the elimination half-lives were in agreement with those previously reported for indomethacin (Alvan et al., 1975) for indoprofen (Caruso et al., 1980, Christophidis & Huskisson, 1982) and for lonazolac (Schrank,

1981). A shorter $t_{1/2}$ (2-3 h) was initially stated for indoprofen (Caruso et al., 1977) when plasma levels were determined only up to 8-10 h after single oral doses. For subjects aged 61-74 years a $t_{1/2}$ of 10.8 h (range 4.5 - 19.8 h), for indoprofen was reported by Lund et al. (1984). Björkman (1985) found that after intravenous administration of indoprofen to surgical patients the half-life for the S(+)-enantiomer was 201 ± 43 minutes, and for the R(-)-enantiomer 187 ± 62 minutes (means $\pm$ SD).

The estimated clearance of indomethacin was found to be in the same range as reported by Alvan et al. (1975).

Prostanoid biosynthesis

Levels of TXB₂-immunoreactive substance

The levels of TXB₂ measured in drug-free serum were in the same range as reported by many other groups, see Table 4. The levels concord well, in spite of variations in assay methods (radio-immunoassay or gas chromatography/mass spectrometry; Pedersen et al., 1983), and origin of antibodies for immunoassays. Variations in incubation times (30 - 120 min) influence the results relatively little (Hall et al., 1984). TXB₂-levels seem to be more dependent on platelet counts in whole blood and on hematocrits and possibly on the volume of blood allowed to clot (Carter et al., 1985). The summary of results from the different groups favours the concept of a normal range of TXB₂ biosynthesis which is common for humans of various ethnic origins.

Data on intraindividual variations in TXB₂-formation are scarce. Specific deficiency of platelet cyclooxygenase (Malmsten et al., 1975) and familial partial platelet thromboxane synthetase deficiency (Defreyn et al., 1981) have been reported. In the present study, one of the subjects on two of six occasions had predose TXB₂-levels below 100 ng/ml, accompanied by a decreased aggregatory response, but no explanation to these variations were found from exogenous factors. Relatively small intraindividual

Table 4

Reference	n	TXB ₂ in serum (ng/ml) mean $\pm$ SD/SEM	Incub time	Notes
1. Patrono et al. 1980a	45	222.4 $\pm$ 81 (SD)	30 min	
2. Defreyn et al. 1981	? 2 1	200-700 (range) 101 and 33 ng/ml, respectively 3.3	60 min	"Normal values" Partial TX-synthase deficiency Healthy control with ASA
3. Viinikka et al. 1982	6	209 $\pm$ 27.1 (SEM)	60 min	
4. Parry et al. 1982	6 6 6	177.7 $\pm$ 30.6 (SEM) 235.5 $\pm$ 39.2 (SEM) 228.3 $\pm$ 22.4 (SEM)	60 min	Pts with angina pectoris
5. Patrignani et al. 1982	33 4x7 5x7	228 $\pm$ 87 (SD) 321 $\pm$ 55 (SD) 299 $\pm$ 128 (SD)	60 min	Healthy subjects
6. Davi et al. 1982	30 20 10	250 $\pm$ 80 (SD) 300 $\pm$ 100 (SD) 420 $\pm$ 160 (SD) (Data from figure)	30 min	Healthy controls Insulin-dependent diabetics Insulin-independent diabetics
7. Weksler et al. 1983	70	324 $\pm$ 32 (SEM)	60 min	Pts for aorto- coronary surgery
8. Thorngren et al. 1983	12	172 $\pm$ 25 (SEM?)	60 min	
9. Davi et al. 1983	8	a) 297 $\pm$ 62 (SD) b) 285 $\pm$ 52.4 (SD)	30 min	

Table 4, continued

Reference	n	TXB ₂ in serum (ng/ml) mean ± SD/SEM	Incub time	Notes
10. Levy et al. 1983	13	229.3 ± 39.5 (SEM)	60 min	Pts with recurrent polyserositis
	10	292.2 ± 53.9 (SEM)		Healthy controls
11. Nuotto et al. 1983	12 x4	(180 - 225) ± (20-30) SEM (from figure)	30 min	
12. Orchard et al. 1983	3	248.4 ± 30.5 (SD)		
13. Fischer et al. 1983	2	180	60 min	Means for each pair of subjects
	2	142		
	2x3	180 - 305		
14. Svensson & Samuelsson 1983	9	290 (from figure)	30 min?	Pts with cerebrovascular disease
15. FitzGerald et al. 1983b	7	a) 435 ± 70 (SD) b) 463 ± 87 (SD)	37°C for 45 min + 20°C for 2 h.	
16. Pedersen et al. 1983	5	Ranges: GC/MS: 256-322 RIA I: 240-335 RIA II: 264-342	?	Citrated blood recalcified
17. Brandt et al. 1984	22	250 ± 17 (SE) 261 ± 19 (SE)	30 min	Arterial blood Venous blood

Table 4, continued

Reference	n	TXB ₂ (ng/ml) mean $\pm$ SD/SEM	Incub time	Notes
18. Toivanen et al. 1984	6	59.7 $\pm$ 13.8 (SD) ng/ 10 ⁸ platelets	60 min	Platelet count: 245 $\pm$ 44 x 10 ⁹ /L
	6	70.5 $\pm$ 25.5 (SD)ng/ 10 ⁸ platelets		299 $\pm$ 90 x 10 ⁹ /L
	6	72.8 $\pm$ 33.3 (SD)ng/ 10 ⁸ platelets		292 $\pm$ 34 x 10 ⁹ /L
19. Hall et al. 1984	5	238 $\pm$ 57 (SD)	120 min	
20. Ciabattoni et al. 1984	20	264 $\pm$ 128 (SD)	60 min	Pts with chronic glomerulonephritis
	19	299 $\pm$ 128 (SD)		Healthy controls
21. Mehta et al. 1984	15	383 $\pm$ 32 (SEM)	60 min	
22. Riess et al. 1984	7	1.10 ng/10 ⁶ platelets (median level)	60 min	Pts with atherosclerotic disease
23. Dahl & Uotila 1984	6	520 $\pm$ 130(SEM)pmol/ml =193 $\pm$ 48 ng/ml	60 min	Drugs or NaCl added in vitro
24. FitzGerald & Oates 1984	5	a)338 $\pm$ 55 (SE) b)310 $\pm$ 73 (SE) c)288 $\pm$ 72 (SE) d)304 $\pm$ 62 (SE)	?	No details on method
25. Pedersen & FitzGerald 1984	5	300 $\pm$ 31 (SD)	45 min?	Missing details on method
	10	276 $\pm$ 45 (SD?)		

Table 4, continued

Reference	n	TXB ₂ in serum (ng/ml) mean $\pm$ SD/SEM	Incub time	Notes
26. Cerletti et al. 1985	12	655 $\pm$ 80 (SEM) _{pmol/ml} = =243 $\pm$ 30 ng/ml	30 min	
27. Weksler et al. 1985	25	229 $\pm$ 32 (SD)	60 min	Pts with stroke or TIA
28. De Caterina et al. 1985a	10	305 $\pm$ 71 (SD?)	?	Pts with AMI
29. Carter et al. 1985	60	228 $\pm$ 88 (SD)	60 min	
30. Alessandrini et al. 1985	177	300 $\pm$ 108.2 (SD)	60 min	
31. Pedersen & FitzGerald 1985	7	313 $\pm$ 57 (SEM?)	?	No details on method
32. Longenecker et al. 1985	8x5	Means (?) ranging 150 - 330 From figure	30 min	50 μ l of 95 % ethanol added in vitro
33. Toivanen et al. 1985	12	241.0 $\pm$ 56.3 (SEM)	60 min	Citrated blood, recalcified. Phosphate buffer added in vitro.
34. Shah et al. 1985	24? 11	182 $\pm$ 11.8 (SEM) 182 $\pm$ 18.6 (SEM)	60 min	Pts with stroke Elderly controls
35. Rao et al. 1985	5	168 pmol (range 94-201)/ 10 ⁸ platelets= 62 ng (range 35-74)/ 10 ⁸ platelets	30 min	

variations in mean TXB₂-levels have been found by other groups in repeated sampling during placebo administration (Patrignani et al., 1982; FitzGerald et al., 1983b; Fischer et al., 1983; De Caterina et al. 1985a).

Reports on TXB₂-biosynthesis in spontaneously clotting blood versus time prior to, and following, a single dose of a drug have been made by others. Compounds studied include aspirin (e.g. Patrono et al., 1980a; Mehta et al., 1984), diflunisal (Patrono et al., 1979), sulfinpyrazone (Patrono et al., 1980b), thromboxane synthetase inhibitors (FitzGerald et al., 1983b; Fischer et al., 1983), tiaprofenic acid (Sorkin & Brogden, 1985), dipyridamole (Patrono et al., 1980b), nitroglycerin (Fitzgerald DJ et al., 1984) and beta-adrenoceptor-blocking drugs (Brandt et al., 1984). Slightly different approaches to stimulate TXB₂ formation in serum were used to evaluate the effects of ibuprofen (Longenecker et al., 1985) and of sulfinpyrazone (Pedersen & FitzGerald, 1985). However, although some of these papers also report drug concentrations in plasma, the correlation with TXB₂-levels have apparently not been investigated.

Levels of 6-keto-PGF_{1α}-immunoreactive substance

The levels of 6-keto-PGF_{1α} found in predose-samples in the present studies were in the same order of magnitude as observed by Parry et al. (1982), Vermynen and Deckmyn (1983) and Davi et al. (1983). They reported that following a dose of dazoxiben, which is an inhibitor of thromboxane synthetase, the levels of 6-keto-PGF_{1α} in serum increased, which favours the idea of shunting AA-metabolism towards PGI₂-formation. However, the immunoreactive material measured is probably a mixture of 6-keto-PGF_{1α} and other PG:s which are formed in clotting blood in much greater amounts (see discussion in paper V). Basal levels of 6-keto-PGF_{1α} in plasma have also been the subject of controversy. The results are extremely sensitive to sampling techniques (Butt & Buchanan, 1983) and are influenced by extraction procedures (Greaves & Preston, 1982) and assay methods (Pedersen et al., 1983; Cerletti et al., 1984). Quantitation of urinary metabolites of PGI₂ seems to

represent the best approach for assessing endogenous synthesis of PGI₂, but does not reveal in which tissue production takes place (FitzGerald et al., 1983a).

In the present studies, assay of PGE₂ or PGF_{2α} in serum would probably have given somewhat higher levels than 6-keto-PGF_{1α} (Parry et al., 1982; Orchard et al., 1983) but essentially the same concentration-response relationships. Rane et al. (1978) measured the release of PGE₂ from thrombin-stimulated platelets from healthy subjects who had ingested indomethacin and found that the degree of suppression correlated with the drug concentration in plasma. From similarly thrombin-stimulated platelets, the release of immunoreactive PGF_{2α} was inhibited by naproxen and correlated inversely with total plasma concentrations of the drug (Tomson et al., 1981). However, in a study by Ekstrand et al. (1981), no correlation was found between inhibition of platelet PGF_{2α}-release and salicylate levels in plasma, nor with the therapeutic response of different doses of ASA alone and in combination with indomethacin. This result accords well with similar reports on spontaneous TXB₂-generation in clotting whole blood after ingestion of ASA.

Platelet aggregation

Platelet aggregation induced by AA

Platelet-rich plasma stimulated by AA produces higher concentrations of TXB₂ than plasma stimulated with ADP, epinephrine or collagen (Siess et al., 1981). It follows that AA-induced aggregation is the most sensitive to inhibition of AA metabolism. However, the aggregatory response to AA is complex, as discussed in IV, V, VI. AA is rapidly metabolized via the cyclooxygenase pathway and endoperoxides, as well as thromboxane A₂, can act proaggregatory (Hamberg et al. 1974, 1975; Patscheke, 1985) via interaction with a common receptor. Stimulation of this receptor seems to induce a burst of events, including release of other proaggregatory substances. The present studies put forward the

hypothesis that the phase of shape change, early in the response to AA, best reflects the activity of cyclooxygenase, or rather the action of formed TXB_2 . This conclusion is supported by observations on the effects of antagonists of the endoperoxide/thromboxane receptor (Patscheke & Stegmeier, 1984; Parise et al., 1984).

The following phases of the aggregatory response probably represent combined actions of proaggregatory AA-metabolites and other substances released from depots in the platelets (Charo et al., 1977). AA-metabolites of the lipoxygenase pathway may also be formed and may interact in the process. The major product of this pathway in platelets is 12-HPETE, which is further converted to 12-HETE, and both these compounds have been demonstrated to inhibit aggregation of human platelets (see Aharony et al., 1984). If formation of 12-HPETE is promoted by blockade of the cyclooxygenase, this could add to the inhibitory effect on platelets. It has also been suggested that AA proper (Jamaluddin & Krishnan, 1985) or a metabolite of AA (Carmo et al., 1985) may antagonize platelet activation via an endoperoxide receptor. Observations on the aggregatory response to AA made in the present study indicate that non steroid anti-inflammatory drugs may interfere differently with the continued response. It cannot be excluded that this reflects additional pharmacological effects within this group of drugs.

Platelet aggregation studies following drug administration

Platelet aggregation following ingestion of a drug has previously been studied by several groups, using various inductive agents and techniques for evaluation of the results. In many cases no attempts have been made to relate the inhibitory effect to concomittant plasma concentrations of the drug.

AA-induced platelet aggregation in relation to plasma concentrations of indomethacin was studied by Rane and coworkers (1978), who measured threshold levels for irreversible aggregation. Pedersen and FitzGerald (1985) recorded the time to reach 50 % increase in light transmission ($\text{LT}_{50} \%$) in AA-aggregation and

related it to a drug activity index of sulfinpyrazone and its active metabolites. A lag-time ($LT_{50} \pm ?$) was also used to evaluate possible effects of nitroglycerin on platelet functions, but the study failed to demonstrate such effects (Fitzgerald DJ et al., 1984). In a study on dipyron, which is a pyrazole derivative like sulfinpyrazone, both threshold values and the extent of AA-induced aggregation were measured, but no correlation was found with plasma levels of dipyron metabolites (Eldor et al., 1984). A minimum plasma drug level of 100 $\mu\text{g/ml}$ of indobufen (derivative of butyric acid) was required for adequate inhibition of collagen-induced aggregation, but half this concentration was needed to inhibit the biphasic response to ADP (Crow et al., 1985). A different approach was employed by Seymour et al. (1984), who found a correlation between plasma aspirin esterase activity and the change in platelet aggregation to collagen after ingestion of aspirin, but not after doses of paracetamol.

FitzGerald et al. (1983c) reported dose-related effects of ASA on the extent of aggregation induced by collagen, epinephrine and ADP. Surprisingly, the ADP-aggregation response returned to normal during a dose-ranging study extended over many weeks, in spite of increasing doses. However, the platelet release reaction appeared to be inhibited throughout the study.

Concentration-effect relationships

There are few reports on relationships between concentrations of antiinflammatory drugs in plasma or synovial fluid and their clinical efficacy. Day et al. (1982) found a relationship between naproxen levels in serum and clinical efficacy. It seems, however, reasonable that the anti-inflammatory response should be related to the concentration of the drugs in the synovial fluid and reports on low levels in severely affected joints may support this concept (Wallis & Simkin, 1983). Kinetic studies of drug concentrations in plasma and synovial fluid show that the latter generally behaves as a "deep compartment", in which the drug level increases slowly (Mäkelä et al., 1981) but also decreases more

slowly than in plasma. If this compartment is the biophase for pharmacological activity in rheumatoid diseases, it seems logical that effects are not correlated with plasma-concentrations, but with the more slowly fluctuating levels in the synovial fluid.

If the effect of non-steroid antiinflammatory drugs is based on their inhibition of the cyclooxygenase enzyme, it should be expected to reach its maximum when the enzyme activity is decreased by 90 % or more. At higher concentrations of the drug, no further effect on prostaglandin-formation would be obtained, and, thus, the effect does not appear to correlate with drug concentrations. If drug concentrations in plasma fall exponentially and if the relationship between logarithm of concentration and effect is sigmoidal, the decrease in effect will be linear with time when drug concentrations are on the linear slope of the sigmoidal curve (Rowland & Tozer, 1980). If there is more than one phase in the log concentration versus time curve, there may also be different phases in the effect-time curve (Figure 7).

If the enzyme cyclooxygenase is of the same structure in all tissues, the affinity constant of a certain drug should be expected to be identical for different tissues. However, drug penetration to the tissues, or into the cells, may vary, so that the concentration of the drug in the close environment of the enzyme is not the same. For example, it has been suggested that the low effect of sulindac on renal PG-synthesis may be due to the lack of active substance in the urine (Bunning & Barth, 1982). Interestingly, potent effects on this organ are exerted by indomethacin, which in part may be tubularly secreted (Helleberg, 1981). On the other hand, diclofenac is excreted unchanged in the urine only to a very small extent (Willis & Kendall, 1978), but is nevertheless highly efficient on ureteral colic, which is considered to be mediated via renal PG production. However, the contribution by active metabolites of diclofenac cannot be excluded.

Thus, the relationships between concentration and response found for the four drugs in the present study are not necessarily valid for other tissues. For fenflumizole, clinical studies are

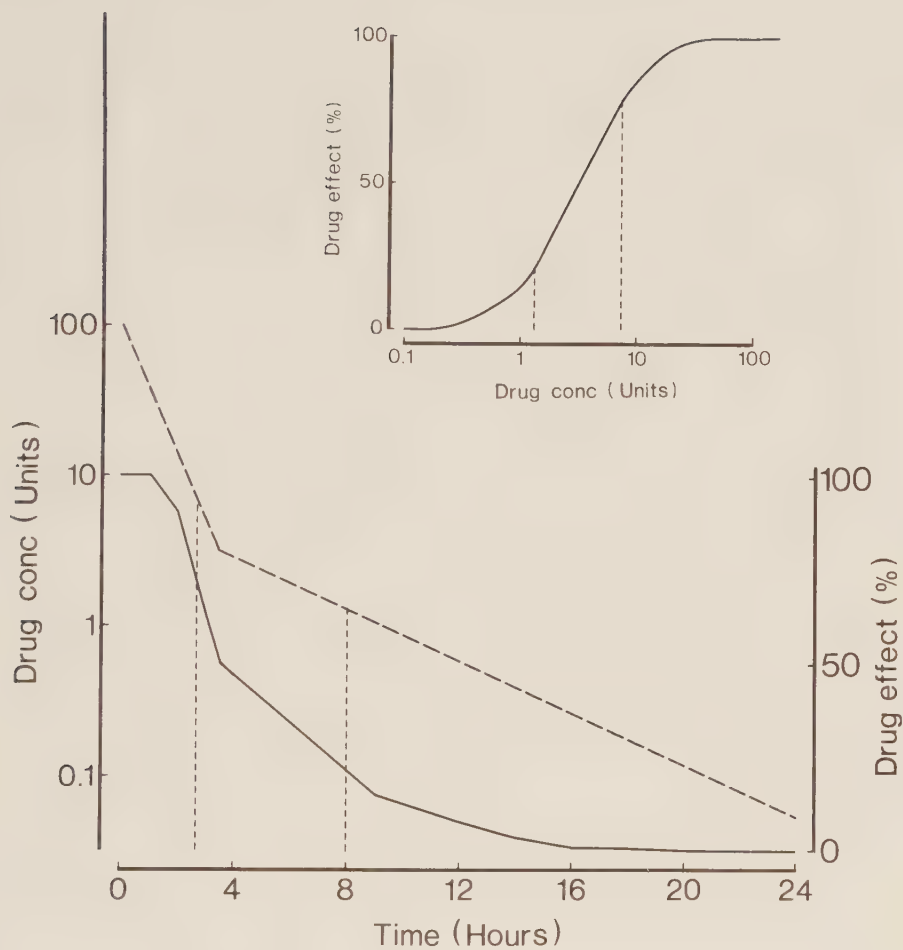


Figure 7. Inserted figure shows the concentration-effect curve of a fictive drug. Large figure shows drug concentration and drug effect as functions of time after a single intravenous dose of the same drug. Note logarithmic scale for drug concentration, but linear scale for drug effect.

underway. For indomethacin, the concentration above which maximum inhibition of TXB_2 -formation was obtained was found to be about $0.5 \mu\text{g/ml}$ which accords with the results of Rane et al., (1978). Interestingly, this is the concentration reached in plasma at steady state at the dosage of $25 \text{ mg} \times 3$ to 5 volunteers reported by Alvan et al., (1975). In 20 patients who also received $25 \text{ mg} \times 3$, the mean steady state concentration was 319 ng/ml , with no difference between responders and non-responders (Baber et al., 1979). When rectal doses were added, the plasma concentration was 467 ng/ml . A concentration of $0.5 \mu\text{g/ml}$ was also found in synovial fluid at 2 h after a single 50 mg dose, and decreased with a $t_{1/2}$ of 9 h, similar to the $t_{1/2}$ found in serum (Emori et al., 1973). Furthermore, for successful closure of persistent ductus arteriosus in preterm infants, it has been suggested that the effective threshold indomethacin level for induction of closure is about $1 \mu\text{g/ml}$ for ten hours postdosing and, for maintenance of the effect, $0.5 \mu\text{g/ml}$ (Seyberth et al., 1983). The results in treatment of ductus arteriosus with indomethacin reported by Yaffe et al. (1980) and Petersen et al. (1981) accord with this suggestion.

Indoprofen has been shown to penetrate well into the synovial fluid, but relatively slowly, and leaves it with a half-life similar to the elimination half-life from plasma (Caruso et al., 1980). The peak concentration reached after an intravenous bolus of 200 mg followed by 100 mg intravenously for 4 hours was $8.31 \pm 1.51 \mu\text{g/ml}$ (mean $\pm$ SEM) and levels $>1 \mu\text{g/ml}$ were maintained for 24 hours (Caruso et al., 1980). In 6 patients given indoprofen 25 mg intraarticularly and 200 mg orally one week later, the synovial fluid concentrations following the oral dose were not different from plasma concentrations during the elimination phase. However, the half-life of the effects was much longer than the concentration half-life of $6.4 \pm 0.7 \text{ h}$ (mean $\pm$ SEM) (Christophidis & Huskisson, 1982).

For lonazolac, concentrations in synovial fluid from fourteen patients who had received $200 \text{ mg} \times 3$ for three days were measured 2 hours after intake of a 200 mg dose. The mean levels were

0.275 µg/ml ($= 8.8 \times 10^{-7}$ M) (Mayrhofer & Thumb, 1984). The time for maximum concentration or the rate of elimination from synovial fluid were not measured.

For naproxen, Tomson et al. (1981) found maximum suppression of platelet $\text{PGF}_{2\alpha}$ -release when plasma-concentrations reached 50-100 µg/ml. Day et al. (1982) reported that 18 of 24 rheumatoid patients with trough serum concentrations of naproxen above 50 µg/ml responded to treatment.

For ASA, a lack of correlation between doses that inhibit platelet functions and those needed for anti-inflammatory effect is obvious (e.g. FitzGerald & Pedersen, 1984a). However, since ASA acts irreversibly as an acetylator, concentration-response relationships are difficult to interpret, but several groups have shown dose-response relationships for platelet TXB_2 -formation with low doses of ASA (Patrignani et al., 1982; FitzGerald et al., 1983c; Nuotto et al., 1983; Weksler et al., 1983; FitzGerald & Oates, 1984; Mehta et al., 1984).

Studies on differential effects by ASA on platelets and endothelial cells have attracted much interest. It was suggested that endothelial cyclooxygenase was less sensitive to ASA and could recover from ASA-inhibition by synthesis of new enzymes (Moncada & Vane, 1978). Results with single doses of ASA administered to normal subjects (Preston et al., 1982) and to patients undergoing aortocoronary by-pass surgery (Weksler et al., 1983) support this hypothesis. Studies with other non-steroid antiinflammatory drugs on human platelets and blood vessels in vitro (Schrör et al., 1980) and platelets and human lung tissue in vitro (Toivanen et al., 1985) have provided further evidence for different sensitivities. However, although inhibition of vascular PG-synthesis was less pronounced than in platelets, and recovery did occur after single doses, repeated doses of ASA 40 mg gave cumulative effects, not only on platelets but also on vein walls (Preston et al., 1982). Also, repeated administration of small aspirin doses reduced urinary excretion of metabolites of both thromboxane and prostacyclin (FitzGerald et al., 1983c). On the other hand,

Sinzinger et al., (1984) reported that daily administration of ASA 1 mg increased platelet sensitivity to extrinsic PGI_2 and PGE_2 . However, in analogy with "up- and down-regulation" of adrenoceptors, this could also indicate decreased exposure to the PG:s in vivo, supposedly by decreased production.

Different sensitivity may be explained by different experimental conditions and by kinetic factors, e.g. penetration of the drug through membranes to reach the target enzyme. Higher concentrations of ASA are needed for inhibition of TXB_2 -formation in plasma in vitro than have been measured ex vivo (Pedersen & FitzGerald, 1984). The same observation was made for ASA-inhibited platelet aggregation induced by collagen (Nitelius et al., 1984). This finding has tentatively been attributed to high concentrations of ASA in the presystemic circulation, where enough platelets should be acetylated to account for the more pronounced effect in vivo.

The slight difference in potency of inhibition of TXB_2 - and 6-keto- $\text{PGF}_{1\alpha}$ -immunoreactivity found with fenflumizole, but not with indomethacin, could indicate selectivity of fenflumizole for cyclooxygenase of different blood cells. Another explanation may be that fenflumizole is bound to, or taken up by, platelets more than by other blood cells from which PGI_2 and 6-keto- $\text{PGF}_{1\alpha}$ are formed, possibly leukocytes, and that drug concentrations in the proximity of the cyclooxygenase enzyme subsequently become different. A presystemic effect does not seem likely, since the concentration-response relationship for fenflumizole was equal after intravenous and oral doses.

Platelets in disease

Platelets are involved in hemostasis and thromboembolic disorders (Fareed et al., 1983; Canadian Cooperative Study Group, 1978; Shah et al., 1985). Vessel injury is associated with altered platelet functions (Kinlough-Rathbone et al., 1983) and this may be the cause of enhanced platelet reactions and shortened platelet

survival in arteriosclerosis (FitzGerald et al., 1984), but platelets may also be involved in the pathogenesis of this disease (Zahavi & Zahavi, 1985; Hess et al., 1985), possibly by the release of the platelet derived growth factor (Deuel & Huang, 1984). Platelet activation may be secondary or contributory to tissue ischemia, e.g. in the brain (Shah et al., 1985) and anti-platelet therapy has been tried in the prevention of stroke (Canadian Cooperative Study Group, 1978) and of myocardial infarctions in patients with unstable angina pectoris (Lewis et al., 1983; Cairns et al. 1985). Platelet hyperreactivity has also been associated with unsatisfactorily regulated diabetes mellitus and this may contribute to the development of vascular complications in patients with this disease (Tindall et al., 1981; Mustard & Packham, 1984).

However, platelets seem to be involved not only in disorders of thromboembolism. Altered platelet functions in hypertension have been studied as a model of smooth muscle (Erne et al., 1984) and of sympathetic neurons (Mattiasson et al., 1979). Defects in platelet uptake and release of serotonin have been associated with migraine (Malmgren et al., 1978, 1980; Launay et al., 1982), and it has even been suggested that migraine is caused by a primary platelet abnormality (Hanington et al., 1981). Increased levels of serotonin in whole blood have been found in patients with carcinoid tumours (Ahlman, 1985) and in patients with untreated coeliac disease (Sjölund & Nobin, 1985), whereas decreased uptake of serotonin was found in blood platelets from depressed patients (Tuomisto & Tukiainen, 1976).

Furthermore, it has been suggested that platelets have an important role in the inflammatory reaction (Ginsberg, 1981), in asthma (Malmgren et al., 1980; Morley et al., 1984; Ameisen et al., 1985) and possibly in cold urticaria (Wassermann & Ginsberg, 1984). Capron et al. (1985) have reported an IgE-mediated cytotoxic effect on parasites (*Schistosoma mansoni*) by platelets and Berrettini et al. (1985) found increased platelet activation in psoriasis. Results from placebo-controlled antiplatelet therapy with dipyridamole and aspirin in membranoproliferative glomerulo-

nephritis Type 1 suggest that progression of the disease could be delayed in the treated group (Donadio et al., 1984).

In addition, platelets appear to have a role in tumor cell metastasis (Honn et al., 1983). Hyperaggregation may be involved in the aetiology of tinnitus (Fabiani et al., 1985). Familial hypercholesterolemia is often accompanied by platelet hyperactivity (Viener et al., 1984), whereas decreased platelet aggregation and prolonged bleeding-time have been observed following a diet rich in eicosapentaenoic acid from fish (Thorngren & Gustafson, 1981; Saynor et al., 1984). Fish also contain great amounts of selenium, and, interestingly, administration of selenium to healthy volunteers prolonged the template bleeding time (Schiavon et al., 1984).

Thus, since platelet functions seem to be altered in a great number of diseases, it is reasonable to assume that drugs which affect platelets in vivo at therapeutic concentrations may interact in pathophysiological processes. It is likely that all antiinflammatory drugs that depress cyclooxygenase activity also affect platelet prostanoid production in vivo, at therapeutic doses. Although the effect of ASA on platelets appears to be beneficial in some disease states, we do not know to what extent suppression of platelet cyclooxygenase activity is desirable (50-90-99 %?). Neither do we know if antiplatelet prostanoids in the vessels can be spared and taken advantage of or if additional effects should be sought. Several biochemical mechanisms other than arachidonic acid metabolism via the cyclooxygenase pathway appear to be involved in normal and pathologic platelet endothelial interactions (e.g. Haslam & Cusack, 1981; Buchanan et al., 1985). This should be considered in the search for efficient and clinically useful antiplatelet drugs. Although it is likely that fenflumizole, as well as the other drugs evaluated in the present study, will impair platelet function, conclusions on the clinical effects on hemostasis should be made with caution until further studies have carried out.

SUMMARY AND CONCLUSIONS

Fenflumizole, a novel non-acidic anti-inflammatory compound, was given to healthy volunteers as single oral doses of 0.1 - 2 mg/kg and as single intravenous doses of 0.1 mg/kg of cold and ^{14}C -labelled compound. Oral doses were rapidly absorbed. Bio-availability was approximately 50 %. After intravenous doses, there was a fast initial decline in plasma concentrations, but the terminal half-life over a one-week period was about 120 hours. The clearance from plasma was 0.55 liters per minute and was almost entirely extrarenal. Labelled compound was more rapidly eliminated from plasma than from blood, which is suggestive of drug binding to blood cells. The calculated value for the volume of distribution at steady state was about 3000 liters. Demethylated and further conjugated metabolites were identified. Renal excretion accounted for about 4 % of the total dose of labelled drug administered intravenously, whereas about 50 % was recovered in the faeces. Enterohepatic circulation as well as extensive tissue binding of the drug is suggested.

In a concentration-dependent manner, fenflumizole suppressed the capacity of endogenous formation of thromboxane B_2 and 6-keto-prostaglandin $\text{F}_{1\alpha}$ immunoreactive substance in blood and prolonged the lag phase in the platelet aggregatory response to exogenous arachidonic acid. These phenomena appeared to be parallel. Other effects on platelet aggregation were more complex, which in part may be due to the bell-shaped concentration-response to arachidonic acid.

In order to obtain reference data from other anti-inflammatory drugs, similar ex vivo studies were conducted after administration of indomethacin (model drug), indoprofen and lonazolac (two newly developed anti-inflammatory drugs) in single oral doses to volunteers. All three compounds inhibited thromboxane generation reversibly and in a concentration-dependent manner, with small differences in potency based on total concentrations in plasma, but all were less potent than fenflumizole. Platelet aggregation was also reversibly inhibited, but differences between the drugs

appeared in the patterns of aggregatory response. It was demonstrated that the degree of inhibition of arachidonic acid-induced aggregation depends on how the aggregatory tracings are evaluated.

It is suggested that the aggregatory response may reflect differences in the mechanisms of action of anti-inflammatory drugs. For comparison of the potential of these drugs to inhibit prostanoid formation in vivo, endogenous thromboxane generation in clotting blood offers a simple and reproducible method. The relationships between effects and drug concentrations in other tissues may be the same as effects on platelets related to plasma concentrations. However, physicochemical properties of each drug will influence drug distribution, and, hence, the relationships between dose and extent and duration of various pharmacological effects.

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On the Pharmacokinetics of Fenflumizole, a Novel Antiinflammatory Agent, after Single Oral Administration to Healthy Subjects

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Abstract: The disposition of fenflumizole, a recently developed imidazole derivative which inhibits prostanoid formation, was studied after single oral administration to eight healthy subjects. Fenflumizole was given in the doses 0.1, 1 and 2 mg/kg as an aqueous suspension. Plasma concentrations within the first 24 hrs were amenable to a two-compartment open model and showed a rapid absorption with a half-life of about 0.5 hr and peak concentrations in plasma after about 1 hr. The elimination phase became dominating after about 10 hrs with a half-life of 14-15 hrs. Peak plasma levels and areas under the concentration-time curves prior to onset of the elimination phase indicated a small reduction of the bioavailability with dose on comparison of the highest and the lowest dose ($P < 0.05$) but for the whole observation period of 24 hrs no dose-dependent bioavailability was revealed. The mean transit time in plasma ranged from 21 to 22 hrs. Two metabolites (demethylation products) were detected in both plasma and urine. Their plasma concentrations amounted to only about 5% of those of the parent compound, and they were rapidly eliminated. The total urinary recovery of the intact fenflumizole and the two metabolites was less than 0.1% of the given doses. The renal clearance of fenflumizole was estimated to about 0.02 ml/hr.

Key-words: Fenflumizole – pharmacokinetics – single oral dosing – antiinflammatory drug – healthy subjects.

Fenflumizole, i.e. 2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole (fig. 1) belongs to the series of substituted triaryl-imidazoles described by Lombardino & Wiseman (1974). Fenflumizole inhibits formation of prostanoids (Corell *et al.* 1983) and in animal models it has analgesic, anti-inflammatory and antipyretic activities comparable to those of other potent inhibitors of cyclooxygenase, e.g. indomethacin (Corell & Hasselmann 1983). However, fenflumizole appears to possess a very low acute gastro-ulcero-genicity and acute toxicity (Corell *et al.* 1983).

A liquid chromatographic method has been developed for determination of fenflumizole concentrations in biological fluids (Midskov 1983). Pharmacokinetic studies on rats showed a rapid oral

absorption with a threefold increase of bioavailability with dose in the dose-range 3-30 mg/kg (Midskov & Arnold, unpublished results). In dogs, the pharmacokinetics following intravenous doses of 3-10 mg/kg appeared independent of dose but otherwise comparable to those of rats. However, after oral administration to dogs the pattern deviated significantly and was incompatible with a compartmental pharmacokinetic analysis. This may be explained by an enterohepatic circulation (Midskov & Arnold, unpublished results). In rats about 15% of the dose was recovered in the urine independent of administration route. In dogs, dosed intravenously, the urinary recovery was reduced to 1-2% of dose.

The present study presents the first data on the

pharmacokinetics of fenflumizole in man, given as single oral doses at three different dose-levels to healthy male volunteers.

Materials and Methods

Subjects. Eight healthy male volunteers participated in the study. Their mean age was 30 years (range 24–41 years), and their mean body weight 71 kg (range 62–83 kg). All subjects were judged to be healthy by clinical examination, electrocardiogram and laboratory tests of haematologic, hepatic and renal functions. No other drugs or alcohol were allowed during investigation days. Each subject gave his written informed consent to participation in the study and the study protocol was approved by the Ethics Committee of the University of Lund, Sweden.

Procedures. On trial days the subjects were fasting from midnight and received fenflumizole in the morning. Each subject ingested fenflumizole in three different doses, 0.1, 1 and 2 mg/kg, on three different occasions separated by at least one week.

Fenflumizole, was synthesized at Dumex Ltd. and aqueous suspensions were prepared (major excipients: Tween 80 and Tragacanth). A suspension with a fenflumizole concentration of 0.5 mg/ml was used for the smallest dose (0.1 mg/kg), whereas a concentration of 5 mg/ml was used for the other two doses (1 and 2 mg/kg). In this way, the volumes of suspension administered to each subject only differed two-fold between the lowest and the highest dose. After each dose the subjects took 100 ml of water, but otherwise no drink or food was allowed until 3 hrs after drug administration. At this time a standardized light meal was served, consisting of bread, milk and cheese. After 4 hrs a regular food-intake was allowed. The subjects were resting in a semi-supine position during the first 4 hrs, and were not allowed to smoke.

Heparinized blood samples were taken prior to and 15, 30 and 60 min., and 1.5, 2, 3, 4, 6, 8, 12 and 24 hrs after drug administration. All samples were protected from light, and allowed to stand at room temperature for 30 min. before centrifugation. Plasma was then collected in plastic tubes (polystyren) and stored up to 4 months at -18° until analysis. During this time degradation of fenflumizole was negligible.

Urine was collected in four intervals 0–4 hrs, 4–8 hrs, 8–12 hrs and 12–24 hrs after drug administration and protected from light. The volumes were recorded and the urine was then kept at -18° until further analysis.

Blood pressure and heart rate were controlled during the first 4 hrs after each dose. Adverse reactions were recorded in a separate protocol, both as spontaneous reports and after active questioning. The laboratory tests mentioned above were repeated about one week after each dose.

Analytical method. The concentration of fenflumizole

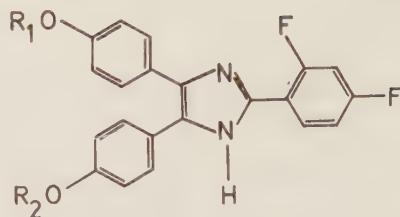


Fig. 1. Chemical structures of a) $R_1 = R_2 = \text{CH}_3$: fenflumizole, b) R_1 (or R_2) = CH_3 and R_2 (or R_1) = H : monodemethyl-fenflumizole and c) $R_1 = R_2 = \text{H}$: didemethylfenflumizole.

in plasma was determined by means of a liquid chromatographic method (assay II, Midskov 1983) which applies fluorescence detection of the column eluate. Two metabolites, chromatographically identified as mono- and didemethyl-fenflumizole (fig. 1) became also visible in the plasma chromatograms. The limit of quantitation was below 1 ng/ml and the coefficient of interassay variation for fenflumizole was below 7% within a concentration range of 50–150 ng/ml. Enzymatic incubation with β -glucuronidase (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was performed.

The urine was analyzed using a modification of the plasma method (Midskov, unpublished results). Briefly, in the reversed-phase chromatography the column temperature was elevated and a third component was added to the mobile phase (tetrahydrofuran). This method also comprises determination of the two metabolites mentioned above.

The possible presence of glucuronide metabolites in urine was also examined. However, following the enzymatic incubation the urine procedure appeared inapplicable due to extraneous chromatographic peaks. This obstacle has not yet been circumvented.

Data processing. Sample concentrations were determined by means of linear plasma standard curves (linear regression, method of least squares).

In characterizing the plasma concentrations measured the data points were fitted to a two-compartment open model. An equation of the form

$$C = C_1 \cdot \exp(-\lambda_1 t) + C_2 \cdot \exp(-\lambda_2 t) - C_a \cdot \exp(-k_a t) \quad (1)$$

was applied to the concentrations observed for each administration and each subject (see table 1 for explanations of the symbols). The individual curve segments were determined manually by the method of residuals in semilogarithmic plots (Gibaldi & Perrier 1975).

The areas under the concentration-time curve were calculated by applying the trapezoidal rule and also by integration of the exponential functions in the pharmacokinetic model. The latter theoretical area is derived from the model and implies a calculation of the area to infinity according to

$$\text{AUC} = \left(\frac{C_1}{\lambda_1} + \frac{C_z}{\lambda_z} - \frac{C_a}{k_a} \right). \quad (2)$$

The time to reach peak plasma concentration ($t_{\max}$) and the maximum plasma concentration reached ($C_{\max}$) were determined from the experimental data. The mean transit time in plasma was conceived as

$$\bar{t} = \left(\frac{1}{\lambda_1} + \frac{1}{\lambda_z} - \frac{1}{k_a} \right). \quad (3)$$

In theory, $\bar{t}$ is defined as the mean time for the intact drug to transit through the organism. Therefore $\bar{t}$ includes absorption into the body as well as all disposition processes. For drugs obeying the kinetics of a two-compartment open model, $\bar{t}$ may be approximated by the reciprocal of the terminal elimination rate constant, i.e. $1/\lambda_z$.

An estimation of the average renal clearance was obtained by

$$\text{CL}_R = \frac{\text{Ae}(\infty)}{\text{AUC}} \quad (4)$$

Here, CL_R is seen to vary inversely with the term C_z/λ_z which admittedly preponderates in the expression for AUC (equation (2)). By relating CL_R or total body clearance to $\bar{t}$ an inverse relationship therefore prevails (equation (3)).

Pharmacokinetic symbols and nomenclature are in accordance with the guidelines of the American College of Clinical Pharmacology (ACCP Nomenclature Report 1982).

Statistical evaluation was performed using Student's *t*-test and the Wilcoxon test for paired differences. A significance level of 5% was applied.

Results

The mean plasma concentration-time curves of fenflumizole after oral administrations of 0.1, 1 and 2 mg/kg are shown in fig. 2.

Data for characterization of the absorption phase are presented in table 2. The maximum plasma concentrations ($C_{\max}$) expressed as mean values $\pm$ S.E.M. were 32 ± 7 ng/ml after the dose of 0.1 mg/kg, 229 ± 34 ng/ml after 1 mg/kg and 390 ± 57 ng/ml after dosage 2 mg/kg. Dose and $C_{\max}$ thus did not appear to be directly proportional. On the basis of intraindividual values a difference ($P < 0.05$) was observed for $C_{\max}$ when comparing the doses 0.1 mg/kg and 2 mg/kg. The individual ratios of $C_{\max}$ for the dose 0.1 mg/kg relative to 2 mg/kg appeared in the range 1.1–2.4, as calculated per dose unit. For all subjects the absorption was fast with mean values for $t_{\max}$ ranging from 1.1 to 1.3 hrs for all three dose-levels. By recognizing that the time to reach the maximum concentration ($t_{\max}$) was almost con-

Table 1.

Pharmacokinetic parameters.

Symbol	Definition	Unit
t	Time	hr/hrs
C	Concentration in plasma at any time t	ng/ml
$t_{\max}$	Time to reach $C_{\max}$	hr/hrs
$C_{\max}$	Maximum plasma concentration	ng/ml
C_1	Zero time intercept of the λ_1 -line	ng/ml
C_z	Zero time intercept of the λ_z -line	ng/ml
C_a	Zero time intercept of the k_a -line	ng/ml
λ_1	Distribution rate constant	hr ⁻¹
$t_{1/2\lambda_1}$	Half-life associated with λ_1	hr/hrs
λ_z	Elimination rate constant	hr ⁻¹
$t_{1/2}$	Half-life associated with λ_z	hr/hrs
k_a	Absorption rate constant	hr ⁻¹
$t_{1/2a}$	Half-life associated with k_a	hrs
$\text{AUC}(0-t)$	Area under concentration-time curve from zero to time t	ng·hr/ml
AUC	Area under concentration-time curve from zero to infinity	ng·hr/ml
f	Fraction of dose absorbed	—
CL_p	Total body clearance from plasma	ml/hr
$\bar{t}$	Mean transit time in plasma	hr/hrs
CL_R	Renal clearance from plasma	ml/hr
$\text{Ae}(\infty)$	Amount of intact drug recovered in urine	ng

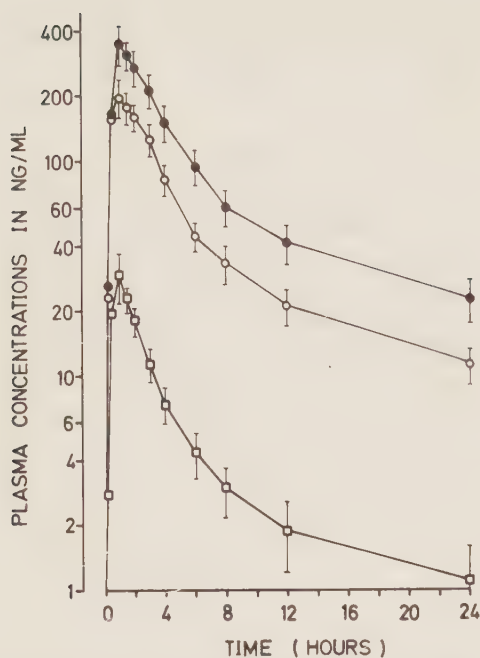


Fig. 2. The mean plasma concentration profiles of fenflumizole after single oral doses of 0.1 mg/kg ($\square$), 1 mg/kg ($\circ$), and 2 mg/kg ($\bullet$) to eight healthy adult subjects. Bars show mean $\pm$ S.E.M.

stant, a relative reduction of the absorption rate is indicated when the dose was increased from 0.1 to 2 mg/kg.

The areas under the concentration-time curves regarding 0–24 hrs are also given in table 2. There seems to be a linear relation to the corresponding doses, indicating dose independence. However, the area under the curve was examined as a function of the time (fig. 3). When relating these trun-

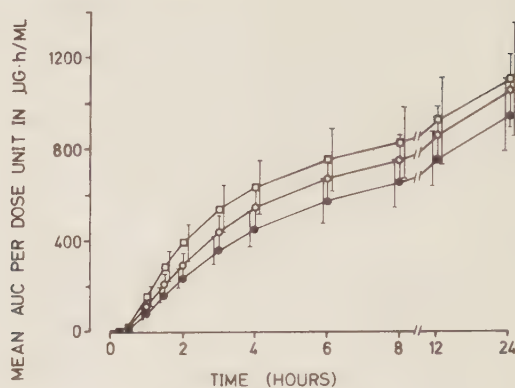


Fig. 3. The mean AUC(0–t) (ng·hr/ml) per dose unit of fenflumizole (mg/kg) versus time *t* after single oral doses of 0.1 mg/kg ($\square$), 1 mg/kg ($\circ$), and 2 mg/kg ($\bullet$) to 8 healthy adult subjects. Bars show mean $\pm$ S.E.M.

cated curve areas applying to the doses 0.1 mg/kg and 2 mg/kg a significant intraindividual difference ($P < 0.05$) appeared for all times up to 12 hrs. The differences were most pronounced at 4–6 hrs ($P < 0.01$) where they ranged 5–70% in favour of the lower dose. (See Discussion).

The plasma concentration-time curves could be described by a two-compartment model. The derived constants from this model are given in table 3. The individual pharmacokinetic parameters revealed only a few minor deviations from the means, and are therefore not presented. The absorption phase described by C_a , k_a , and $t_{1/2a}$ is characterized by values of $t_{1/2a}$ of about 0.5 hr for all three doses. The succeeding phase supposedly reflecting mainly distribution (from about 2 to 8 hrs after dosing) had a mean half-life $t_{1/2\lambda 1}$ ranging from 1.2 to 1.6 hrs for the doses used.

Table 2.

The measured values of the maximum plasma concentration reached (C_{max}), the time to reach C_{max} (t_{max}) and the 24 hrs area under the concentration-time curve (AUC(0–24 hrs)) following single oral doses of fenflumizole to eight healthy subjects.

Doses	0.1 mg/kg		1 mg/kg		2 mg/kg	
Parameter	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
C_{max}	31	7	229	34	390	57
t_{max}	1.1	0.1	1.1	0.2	1.3	0.3
AUC(0–24 hrs)	109	24	1048	157	1878	325

Table 3.

Model dependent pharmacokinetic parameters derived from the two-compartment open model for single oral doses of fenflumizole to eight healthy subjects.

Doses	0.1 mg/kg		1 mg/kg		2 mg/kg	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Parameter						
C_1	57	11	331	49	718	151
λ_1	0.62	0.04	0.47	0.04	0.51	0.08
$t_{1/2\lambda 1}$	1.2	0.07	1.5	0.1	1.6	0.2
C_z	3.4	1.3	39	8	73	15
λ_z	0.05	0.003	0.05	0.004	0.05	0.006
$t_{1/2}$	13.9	0.9	13.8	1.3	15.1	1.8
C_a	62	12	373	51	849	189
k_a	1.5	0.14	1.7	0.2	1.4	0.1
$t_{1/2a}$	0.48	0.05	0.47	0.05	0.51	0.05
AUC	121	34	1265	212	2398	479
$\bar{t}$	20.9	1.3	21.6	2.2	22.2	2.6

The elimination phase was found to dominate after about 8 to 10 hrs. The half-life $t_{1/2}$ was calculated on the basis of observations from 12 to 24 hrs, although this period only comprises two sampling times. For all three doses, the mean values of $t_{1/2}$ are 13.8 to 15.1 hrs (table 3), with S.E.M. being about 10%. However, it must be emphasized that with only two data points in the elimination phase, the basis of calculation becomes weak.

The mean values of the mean transit time in plasma, ($\bar{t}$, table 3) were 20.9 (0.1 mg/kg), 21.6 (1.0 mg/kg), and 22.2 hrs (2.0 mg/kg), with S.E.M. values ranging from 6 to 12%. No dose-dependent differences were thus seen for $\bar{t}$.

Mono- and didemethylated fenflumizole could also be identified in the chromatograms. Plasma analyses of the two metabolites showed most frequently concurrent plasma concentration profiles analogous to that of the parent drug, i.e. with t_{max} of about 1 hr. C_{max} were low with approximate mean values for both compounds of 2–12 ng/ml, increasing with dose. In plasma, deconjugation treatment with the aid of β -glucuronidase did not reveal any glucuronide metabolites.

Fenflumizole and the two metabolites were also detected in the urine. The amounts recovered within 24 hrs were below 0.1% of the administered dose. Most frequently about half the amount was excreted within the first collection period 0–4 hrs, independent of dose. An estimate of the renal

clearance of fenflumizole was obtained from equation (4), giving a value of about 0.02 ml/hr.

All the oral doses were well tolerated by the subjects and no adverse reactions to fenflumizole occurred, neither clinically nor in laboratory tests.

Discussion

The present study demonstrates that the 0–24 hrs plasma concentration profiles of fenflumizole following oral doses of 0.1–2 mg/kg can be described by a two-compartment open model.

Fenflumizole appears to be rapidly absorbed with the dosage form studied (aqueous suspension). The measured peak time (t_{max}) varied between 0.5 and 1.5 hrs with one exception of subject 7, dose 2 mg/kg, where t_{max} was 3 hrs. This subject was further distinguished at this dose by displaying the lowest C_{max} , being as low as one third of the mean.

The concept of dose-independent kinetics is featured by coincidental concentration profiles of the doses involved, calculated per dose unit. Fig. 3 depicts mean AUC(0– t) divided by the dose as a function of the time t until the last sampling time (24 hrs) for each of the doses. The plotting of these truncated areas versus time may serve to elucidate both the rate and extent of drug disposition. The order of the mean curves could reflect that the systemic bioavailability relative to dose tends to be higher after doses of 0.1 mg/kg than

after the two higher doses. This is consistent with reaching a mean value for $C_{\max}$, which, as mentioned above, had also been reduced relative to dose. In keeping with this, it was found that $C_{\max}$ and the truncated area curves for each subject demonstrated no significant difference as regards the doses (mg/kg) 0.1 versus 1 and 1 versus 2. However, as mentioned above, a significant intraindividual difference between doses of 0.1 mg/kg and 2 mg/kg appeared for both parameters early in the observation period. The non-linearity of $C_{\max}$ with dose did not become evident from the pharmacokinetic parameters derivable from the model. This can readily be explained by the approximative character of the estimation procedure. Concerning the truncated curve areas, maximum intraindividual differences between the dose 0.1 mg/kg and 2 mg/kg occurred at 4–6 hrs, i.e. prior to onset of the elimination phase. However, the differences did not persist at the end of the 24 hrs observation period. In accordance with the latter finding no statistical difference was found between the two theoretical areas to infinity (AUC).

The plasma clearance of fenflumizole (CL_p) cannot be calculated until data on intravenous administration are available. However, the question of dose dependence may be approached on the basis of the oral data presented here. CL_p can be expressed as $(\text{Dose}/\text{AUC}) \cdot f$, where f is the oral availability. On account of the seemingly dose-independent bioavailability over the whole observation period, the plasma clearance of fenflumizole should also be independent of the three doses chosen.

Both metabolites were in most cases detectable in plasma around $t_{\max}$ of the parent drug. The concentrations amounted to only about 3–6% of the corresponding fenflumizole plasma concentrations. These low levels may suggest a rapid plasma turn-over of the metabolites. No glucuronides of the two metabolites were detected in plasma.

The results of the urine analyses revealed a very small urinary excretion rate and a low renal clearance. This indicates that excretion routes other than the renal are preferred. In rats and

dogs given isotope-labelled drug the radioactivity was mainly recovered in the faeces suggesting excretion from the liver or the gut wall as the principal elimination route for unchanged drug and metabolites (Midskov & Arnold, unpublished results). This may also be the case in man.

In conclusion, fenflumizole administered orally to healthy adults is rapidly absorbed and reaches a peak plasma concentration after about 1 hr. The elimination becomes dominating after about 10 hrs and occurs with a half-life of about 15 hrs. A decreased bioavailability with dose is visible early in the observation period, as measured by $C_{\max}$ and truncated area curves. Two metabolites appear in low amounts in plasma and are rapidly cleared. The rate of elimination of fenflumizole from plasma seems independent of dose and so does the plasma clearance. Urinary recovery of fenflumizole and the metabolites amounts to less than 0.1% of the administered dose, independent of dose. No obvious adverse reactions to the fenflumizole formulations could be noted.

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Single Intravenous and Oral Doses of Fenflumizole: Pharmacokinetics and Effects on Prostanoid Formation

By

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Abstract: Fenflumizole is a substituted triaryl-imidazole with anti-inflammatory activity. Its disposition in man was studied after single intravenous and oral doses to 8 healthy male volunteers. Formation of the prostanoids thromboxane B₂ (TXB₂) and 6-keto-prostaglandin F_{1α} (6-k-PGF_{1α}) in clotting blood was studied concomitantly. The pharmacokinetics after intravenous doses (0.1 mg/kg) could be fitted to a three compartment model and the half-lives (t_{1/2}) corresponding to the three phases were 2 min., 1 hour and 15 hours, respectively. The volume of distribution was 386 l and the plasma clearance 0.5 l per min. Oral doses (0.5 mg/kg) were rapidly absorbed (t_{1/2} = 0.2 hr) and the following elimination from plasma had two phases with half-lives of 1 hour and 14 hours. Bioavailability was 50% due to a pronounced first-pass effect. The two metabolites mono- and didemethylfenflumizole were detected after both oral and intravenous doses and their maximum plasma concentrations occurred after 1-2 hours irrespective of the administration route. A concentration dependent depression of prostanoid formation was seen, the IC₅₀ for TXB₂ and 6-k-PGF_{1α} being 19 and 53 ng/ml respectively.

Key-words: Fenflumizole – pharmacokinetics – single intravenous dosing – single oral dosing – prostanoids.

Fenflumizole is a substituted triaryl-imidazole (fig. 1), which has the activity of an antiinflammatory and analgetic compound in animal models (Corell *et al.* 1983; Corell & Hasselmann 1983). Although its toxicity appears to be considerably lower than that of many other antiinflammatory substances (Corell *et al.* 1983), its inhibitory activity on prostanoid formation seems to be comparable to or more potent than that of indomethacin, as shown in laboratory rodents (Corell *et al.* 1983) and in human whole blood *ex vivo* (Vinge *et al.* 1984; Vinge 1985).

Fenflumizole has previously been studied in man after oral doses at different dose-levels (Mid-

skov *et al.* 1984). The pharmacokinetic profile could be described by a two-compartment open model with a rapid absorption phase and an elimination phase with a half-life of about 15 hours. Over the dose-range 0.1–2 mg per kg body-weight the bioavailability appeared to be constant. In plasma two metabolites were detected which were rapidly cleared and only reached low concentrations as compared with the mother compound.

The aim of the present study was to obtain further data on the pharmacokinetics of fenflumizole by studying its disposition after single intravenous doses to man, and relate it to the kinetics after oral administration. It was also of interest to study whether different administration routes would influence the relationship between plasma

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concentrations of the drug and the degree of prostanoid formation in blood.

Materials and Methods

Subjects. Written informed consent to participation was obtained from 8 healthy men, whose ages ranged 25–42 years (mean 31 years) and body-weights 60–88 kg (mean 71.9 kg). All of them had participated in a previous study on the pharmacokinetics of fenflumizole after single oral doses (Midskov *et al.* 1984). They all passed a clinical health examination including laboratory tests of haematological, hepatic and renal parameters before entering the study. They had not taken any drugs during one week before each dose of fenflumizole, and abstained from alcohol during 24 hours prior to and through each trial day.

Procedure. Fenflumizole was given to all subjects in an intravenous dose (0.1 mg/kg) and an oral dose (0.5 mg/kg) with at least 6 days between the doses. Both doses were given in the morning, and the subjects had been fasting since midnight. They rested in a supine or semirecumbent position during the first 3–4 hours. A standardized breakfast was served at 3 hours after dose administration. After still another hour there were no restrictions in the diet except for alcohol. Smoking was not allowed during the first 4 hours after each dose. The subjects were asked to avoid hard physical exercise on trial days.

The intravenous dose of fenflumizole was given in an aqueous solution of 2 mg/ml (major excipients: Carbowax 300, propylene glycol and hydrochloric acid) as a short infusion over 5 min. into an antecubital vein (in one case into a vein on the wrist).

For the oral administration an aqueous suspension of fenflumizole 2 mg/ml was used (major excipients: Tween 80 and tragacanth). An appropriate volume of the suspension was given to the subjects, precedent to 100 ml of water.

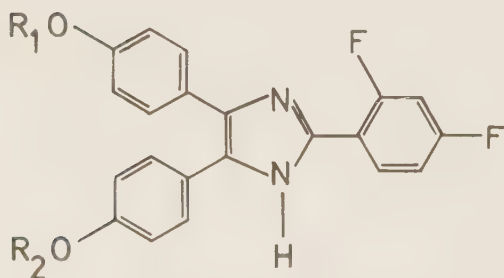


Fig. 1. Chemical structure of fenflumizole ($R_1=R_2=CH_3$) and its two metabolites: Monodemethyl-fenflumizole (R_1 (or R_2)=H; R_2 (or R_1)= CH_3) and didemethyl-fenflumizole ($R_1=R_2=H$).

For determination of drug concentrations in plasma venous blood was sampled in heparinized tubes (Venject®) immediately prior to each drug administration and after 15 and 30 min. and after 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24 and 28 hours. After the intravenous dose additional blood samples were collected at the end of the infusion (=0 hours) and at 5 and 45 min. after the end of the infusion. All blood samples were kept protected from light at room temperature for 30 min. before centrifugation. Plasma was stored at -18° until analysis.

For measurement of prostanoid formation blood was collected in siliconized tubes (Vacutainer®) prior to both doses, and at 15 minutes and 1, 2, 6 and 24 hours after. A sample was also collected immediately after the intravenous drug infusion. The blood was allowed to clot at 37° for one hour, then centrifuged for separation of serum which was kept in polypropylene caps at -18° until analysis (Patrino *et al.* 1980).

Drug analysis. Fenflumizole concentration in plasma was determined by means of high performance liquid chromatography (HPLC) applying fluorescence detection of the column eluate (Midskov 1983).

Two metabolites, identified as mono- and didemethyl-fenflumizole (fig. 1), were also extracted from plasma, but did not interfere with the fenflumizole assay. No interference from endogenous sample constituents was seen. The detection limit was below 1 ng/ml.

The accuracy of the method was estimated by the day to day variations (relative standard deviation) of the calibration curve: Slope variation 8%, linear correlation coefficient variation 4%.

Day to day variations (relative standard deviation) of samples with known drug concentrations (precision) was below 7%, decreasing with rising drug levels.

Analysis of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$. Serum samples were assayed for their contents of immunoreactive thromboxane B_2 (TXB₂) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF_{1 α}) by use of two commercially available radioimmunoassays (3H -TXB₂ and 3H -6-keto-PGF_{1 α} -RIA, New England Nuclear, Boston, Ma, U.S.A.). For TXB₂-measurements serum was diluted 1:100 with 0.05 M phosphate buffer pH 7.4. 6-keto-PGF_{1 α} -like activity was measured in undiluted serum. Standards were prepared in buffer but serum was added to the incubation mixture in the same volume as in the unknown samples. Limits of detection were 5 ng/ml in the TXB₂-assay and 0.1 ng/ml in the 6-keto-PGF_{1 α} -assay. Cross-reactivity of TXB₂ in the 6-keto-PGF_{1 α} -assay was <0.1% or below detection limit when TXB₂-concentrations were 500 ng/ml or lower (Vinge 1985).

Data processing. The plasma concentrations measured in the intravenous study were fitted to a three compartment open model with first order elimination from the central compartment. The equation was:

$$C = C_1 \cdot \exp(-\lambda_1 t) + C_2 \cdot \exp(-\lambda_2 t) + C_3 \cdot \exp(-\lambda_2 t)$$

For explanations of the symbols see table 1. The nomenclature is in accordance with the guidelines of the American College of Clinical Pharmacology (1982).

Table 1.

Pharmacokinetic parameters.

Symbol	Definition	Unit
t	Time	hr/hrs
C	Concentration in plasma at any time t	ng/ml
t _{max}	Time to reach C _{max}	hr/hrs
C _{max}	Maximum plasma concentration	ng/ml
C ₁	Zero time intercept of the λ_1 -line	ng/ml
C ₂	Zero time intercept of the λ_2 -line	ng/ml
C _z	Zero time intercept of the λ_z -line	ng/ml
C _a	Zero time intercept of the k _a -line	ng/ml
λ_1	Distribution rate constant 1	hr ⁻¹
t _{1/2λ_1}	Half-life associated with λ_1	hr/hrs
λ_2	Distribution rate constant 2	hr ⁻¹
t _{1/2λ_2}	Half-life associated with λ_2	hr/hrs
λ_z	Elimination rate constant	hr ⁻¹
t _{1/2}	Half-life associated with λ_z	hr/hrs
k _a	Absorption rate constant	hr ⁻¹
t _{1/2a}	Half-life associated with k _a	hrs
AUC(0-t)	Area under concentration-time curve from zero to time t	ng · hr/ml
AUC	Area under concentration-time curve from zero to infinity	ng · hr/ml
f	Fraction of dose absorbed	—
CL _p	Total body clearance from plasma	ml/hr
V _{ss}	Volume of distribution at steady state	l
V _c	Volume of distribution of central compartment	l

The constants were fitted to individual data by non-linear least square regression. The procedure was a modified Gauss-Newton method with numeric evaluation of the gradients in the single points (Feldman 1982). The program was written in Algol for a RC-8000 computer.

For the oral study, the model was reduced into a two-compartment open model with first order absorption and elimination.

Areas under plasma concentration curves (AUC), values for plasma clearance and other pharmacokinetic parameters were derived from the above mentioned constants, after calculation by standard pharmacokinetic methods.

Adverse effects. Blood pressure and heart rate were measured at blood sampling times during 4–6 hours after each dose. Adverse effects, reported spontaneously by the subjects as well as at an interview after each sampling period, were noted in a separate protocol. Laboratory tests were checked 2 days after each dose.

The protocol of the study was approved of by the Ethics Committee at the Medical Faculty, University of Lund, Sweden.

Results

Plasma concentrations and pharmacokinetics.

The mean plasma concentrations of fenflumizole in the intravenous and oral studies are shown in

fig. 2. The maximum plasma concentrations were 160–694 ng/ml in the intravenous study at time 0, and 107–419 ng/ml in the oral study in which they occurred from 30 min. to 2½ hours after dosage.

For one subject the plasma concentration curves

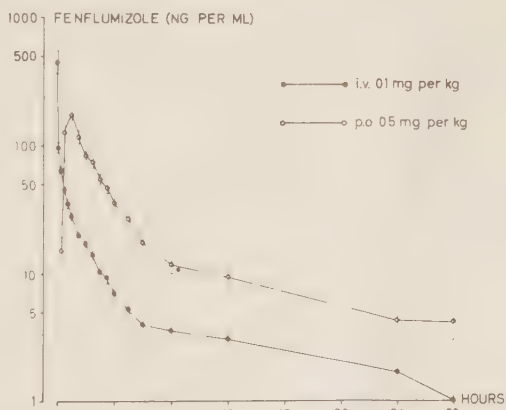


Fig. 2. Plasma concentrations of fenflumizole (mean $\pm$ S.E.M.) after intravenous (0.1 mg/kg) and oral (0.5 mg/kg) administration to seven male volunteers.

could not be well fitted to the model. Technical difficulties in blood sampling may have contributed to the divergent result after the intravenous dose. However, he also showed an irregular plasma concentration pattern after the oral dose. He was therefore omitted from the kinetic part of the results, but the results from the measurements of prostanoids contain data from all eight volunteers.

The mean plasma concentrations of the two metabolites, mono- and didemethyl fenflumizole are shown in fig. 3. Ranges of the maximum metabolite levels following oral administration were 2.0–11.2 ng/ml and 0.2–8.1 ng/ml for the mono- and didemethyl compound, respectively. Following intravenous administrations (of a 5-fold lower dose) the metabolite concentrations were near the detection limit, and di-demethyl fenflumizole was not measurable in three volunteers. Maximum mono-demethyl fenflumizole levels ranged between 0.3 and 1.1 ng/ml.

The pharmacokinetic constants derived from analysis of the fenflumizole data are listed in table

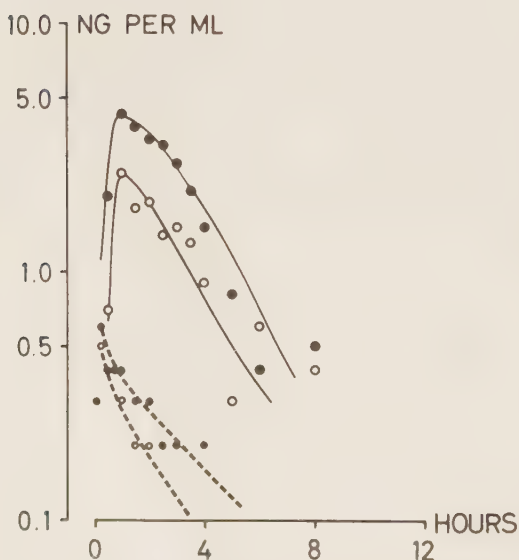


Fig. 3. Mean plasma concentrations of the two fenflumizole-metabolites: Monodemethyl-fenflumizole (●) and di-demethyl-fenflumizole (○) in seven male volunteers after intravenous (0.1 mg/kg, ----) and oral (0.5 mg/kg, —) administration of fenflumizole.

2. It should be noted that the three compartment model, which fitted the intravenous data could not be applied to the oral study due to the short, first distribution phase ($t_{1/2} \approx 0.03$ hrs ≈ 2 min.). The fenflumizole half-life in the next distribution phase was the same in both studies (≈ 1 hr) and this observation was true also for the terminal elimination half-life (≈ 15 hrs). The absorption rate constant, k_a , corresponded to an absorption half-time of 0.2 hrs ≈ 12 min.

The oral bioavailability, i.e. the fraction of a dose absorbed unmetabolized (f) was fifty per cent, provided a linear relationship between dose and AUC is assumed in the dose interval in question. In fig. 4 the AUC's versus doses are shown for four dose-levels investigated in the same subjects. The oral doses 0.1, 1 and 2 mg/kg are taken with permission from Midskov *et al.* (1984).

The plasma kinetics of the two metabolites (fig. 3) could not be examined as detailed as those of the parent compound. The data were insufficient in number and the analytical reliability diminished on approaching the analytical limit of detection. The measured model-independent parameters are given in table 3. The maximum plasma concentrations of both metabolites were observed 1–2 hours following the fenflumizole dose and the AUC's of the metabolites amounted to 0.4–3.9 per cent of the corresponding AUC's of fenflumizole.

Formation of TXB_2 and 6-keto-PGF $_{1\alpha}$.

The mean concentration $\pm$ S.E.M. of TXB_2 -like activity after whole blood clotting before the intravenous dose was 235 ± 22 ng/ml (range 124–295 ng/ml) and before the oral dose 217 ± 38 ng/ml (range 75–425 ng/ml). After both administrations inhibition of the TXB_2 -formation was concentration dependent. The relations between fenflumizole plasma concentrations and TXB_2 -formation expressed as % of predose-levels, are shown in fig. 5a and b. The results from the two administrations could be estimated to form superimposable curves with an IC_{50} of 19 ng/ml.

The mean concentration of 6-keto-PGF $_{1\alpha}$ -like activity in the same sera was before the intravenous dose 0.43 ± 04 ng/ml (range 0.26–0.64 ng/ml) and before the oral dose 0.40 ± 04 ng/ml (range 0.15–0.54 ng/ml). The relations between

drug concentrations and 6-keto-PGF_{1 α} -levels expressed as % of predose-levels are presented in fig. 5c and d. In those samples where no detectable immunoreactivity was present, corresponding to maximum fenflumizole concentrations, the mean % value of the limit of detection has been marked in the plot, and S.E.M. has not been calculated. The results show that the concentration response relationships after the two administrations were similar. The IC₅₀ determined graphically was 53 ng/ml.

Adverse effects.

No changes in blood pressure or heart rate attributable to actions of fenflumizole could be observed. Slight gastrointestinal discomfort was reported by three subjects: at 2 hours after the oral dose together with hunger, at 10 hours after the intravenous dose and immediately after the intravenous dose, respectively. The latter subject also reported a taste sensation for 10 sec. during dose injection resembling the taste of the fenflumizole suspension. The subject who received the intra-

venous dose in a vein at the wrist felt a slight local irritation during the injection, but no lasting effect. One subject reported tiredness, another had slight headache in the afternoon. With the exception of the taste sensation and the slight local irritation all reported effects were judged as non-specific. Local irritation could be attributed to the compounds of the vehicle.

Discussion

The pharmacokinetics of fenflumizole after intravenous administration to man was well described by the three compartment open body model (table 2). The first distribution phase, however, was so short, that omission of the first blood sample, taken at the end of the administration, would have resulted in a description without this phase. The volume of distribution of the central compartment (V_c) was consequently calculated on a minimal data basis, which was also reflected in the substantial variability. The mean volume (11.2 l) covered individual volumes from 3 to 24 l. Clearly

Table 2.

Fenflumizole to seven healthy male volunteers: Pharmacokinetic parameters derived from computer aided fitting of data points to either a three compartment (i.v.) or a two compartment (p.o.) open model.

Parameter	(unit)	Fenflumizole dose					
		0.1 mg/kg i.v.			0.5 mg/kg p.o.		
		Mean	$\pm$	S.E.M.	Mean	$\pm$	S.E.M.
C_1	(ng/ml)	379		82		—	
λ_1	(hr ⁻¹)	26.4		3.2		—	
$t_{1/2\lambda_1}$	(hrs)	0.03				—	
C_2	(ng/ml)	57		5	432		94
λ_2	(hr ⁻¹)	0.78		0.04	0.68		0.08
$t_{1/2\lambda_2}$	(hrs)	0.88			1.02		
C_z	(ng/ml)	5.1		0.6	14.5		3.0
λ_z	(hr ⁻¹)	0.046		0.005	0.051		0.012
$t_{1/2\lambda_z}$	(hrs)	15			14		
C_a	(ng/ml)		—		1043		496
k_a	(hr ⁻¹)		—		3.5		1.0
$t_{1/2\lambda_a}$	(hrs)		—		0.2		
AUC	(ng · hr · ml ⁻¹)	233		33	583		83
f			—		0.49		0.04
CL _p	(ml/hr)	32280		3480		—	
V_c	(l)	11.2		3.2		—	
V_{ss}	(l)	386		44		—	

this individual range was connected with the concentration in the first blood sample which was drawn at a time of rapid concentration changes of fenflumizole. The significance of the first phase can be discussed, but the reflection of mere mixing and distribution within the central compartment is one possible explanation.

The two following phases, showing plasma half-lives of one and fifteen hours agreed well with the previous reported profiles of orally administered fenflumizole (Midskov *et al.* 1984).

The volume of distribution of the peripheral compartment was of an order of magnitude to suggest considerable tissue distribution. It is known from animal studies with ^{14}C -fenflumizole that adipose tissue and skin are the organs retaining radioactivity for the longest time. If man shows the same distribution pattern, a very slow terminal elimination phase can be expected. This would not normally be discovered in single dose experiments, but after repetitive dosing.

Clearance of fenflumizole from the blood stream was fast (≈ 0.5 l/min.) and mostly due to metabolism, biliary or intestinal excretion. Only very small amounts ($<1\%$) of the un-

changed drug are excreted in urine after oral administration to man (Midskov *et al.* 1984). A low renal clearance of fenflumizole has also been found in several animal species.

Oral administration of fenflumizole in suspension gave rise to a fast absorption of the drug with a peak plasma concentration occurring one hour after drug intake. This is in good accordance with previous observations, and also the distribution and elimination half-lives were the same as reported for other oral doses of fenflumizole (Midskov *et al.* 1984). The present study was carried out with the same subjects one year later. The data thus indicate that disposition of fenflumizole shows a small intraindividual variation within healthy subjects.

There appeared to be a dose independent absorption of the drug over a wide dose range: In fig. 4 the AUC's versus doses are shown from 0.1 to 2 mg per kg, and within this range no aberrations from a linear correlation could be seen.

The oral availability of fenflumizole was fifty percent (table 2). A significant first-pass effect rather than faulty absorption is responsible for the low bioavailability:

The plasma clearance is practically identical with the hepatic clearance, since the renal clearance is extremely low. The hepatic extraction ratio can thus be expressed as Cl/Q , when Q is the hepatic blood flow (in normal man 1.2 l per min.). The maximum anticipated oral availability F is given by one minus the hepatic extraction ratio

$$F = 1 - \frac{0.54}{1.2} = 0.55$$

Thus the maximum oral availability is 55%, which is in good agreement with the results reported in this study.

The drug cleared by the liver is either metabolized or excreted into the bile. Ongoing studies with radiolabelled fenflumizole will provide answers concerning metabolites and fecal excretion. In the rat a dose dependent first-pass effect has been demonstrated covering dose levels of 3–30 mg per kg. In this species fenflumizole was excreted into the bile (Arnold, unpublished results). In the dog, the major part of an intravenous ^{14}C -fenflumizole

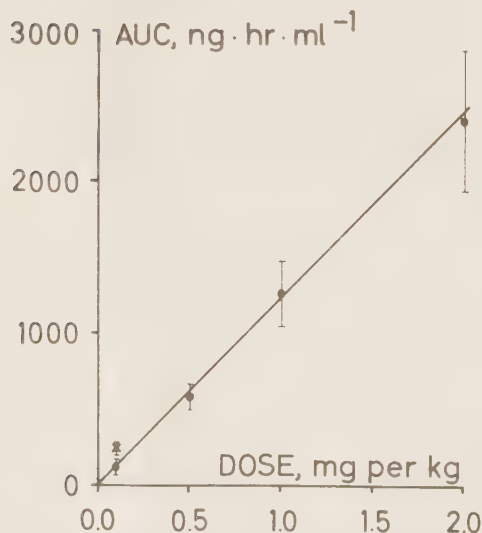


Fig. 4. Areas under fenflumizole plasma concentration curves (mean $\pm$ S.E.M.) versus fenflumizole dose (● oral, × intravenous) in healthy male volunteers. Oral doses of 0.1, 1 and 2 mg/kg from Midskov *et al.* (1984).

dose was recovered in faeces (Arnold, unpublished results).

The two known metabolites, the mono- and didemethylated fenflumizole were present in small amounts in plasma following both administration routes (table 3, fig. 3). Higher levels of the metabolites were detectable in plasma after the oral dose than expected from the intravenous dose, but the metabolite amounts following intravenous administration were at the limit of detection and hence quantified with less precision.

Despite the low metabolite concentrations some general observations can be made: Both metabolites were generated shortly after administration of the parent compound and the didemethylated metabolite was present in smaller amounts than the monodemethylated compound. There were no signs of accumulation of the metabolites, which seemed to be eliminated from the blood stream with the same rate as fenflumizole itself. This shows that the generation rather than the elimination of the metabolites was a rate limiting factor.

The concentration response curves on TXB₂- and 6-keto-PGF_{1 α} -immunoreactivity in serum concorded closely with the results obtained after an oral dose of 1 mg/kg (Vinge *et al.* 1984). It is noticeable that the antibodies in the 6-keto-PGF_{1 α} -assay have been changed by the manufacturer in order to further reduce the cross reactivity towards other prostanoids. This was reflected by lower predose-levels of 6-keto-PGF_{1 α} -immunoactivity in the present study, which implied that the limit of detection was as high as 20–30% of the

predose-levels in several of the cases. More than 70–80% inhibition could thus not be followed, and the degree of inhibition of 6-keto-PGF_{1 α} -formation at drug concentrations > 100 ng/ml was uncertain. Also, it must be born in mind that cross reactivity towards prostanoids like PGE, PGF and PGD may still account for parts of the levels measured in the present study (Pitzke *et al.* 1983; Pedersen *et al.* 1984). The circulating levels of 6-keto-PGF_{1 α} are believed to be much lower, but some formation does probably take place in blood *ex vivo*, possibly by leukocytes (Orchard *et al.* 1983). The predose levels of TXB₂ in serum were considerably higher and correspond well with those reported by other groups (Patrono *et al.* 1980, Weksler *et al.* 1983; Orchard *et al.* 1983; Parry *et al.* 1982; Hall *et al.* 1984).

The values for IC₅₀ of fenflumizole on formation of the prostanoids are very close to those reported in the previous study (Vinge *et al.* 1984). The present results thus seem to confirm a two- or threefold potency difference of fenflumizole on the synthesis of different types of prostanoids. For indomethacin, however, the IC₅₀-values for these effects appear to be identical, which should be expected from a cyclooxygenase inhibitor (Vinge 1985). These findings might reflect different affinity or access of fenflumizole to cyclooxygenase enzymes in different cell-types or tissues (Corell *et al.* 1983). Another explanation might be that fenflumizole, which is a derivative of imidazole, at low concentrations can act selectively on the thromboxane synthetase like imidazole itself and other imidazole-derivatives, e.g. dazoxiben (Parry

Table 3.

Maximal plasma concentrations, time for C_{max} and areas under plasma curves for two fenflumizole metabolites after administration of fenflumizole to seven healthy volunteers.

Dose		Fenflumizole dose							
		0.1 mg/kg i.v.				0.5 mg/kg p.o.			
Metabolite		Mono-demethyl-fenflumizole		Didemethyl-fenflumizole		Mono-demethyl-fenflumizole		Didemethyl-fenflumizole	
Parameter	(unit)	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
C _{max}	(ng/ml)	0.6	0.1	0.5	0.3	4.8	1.2	2.9	1.0
t _{max}	(hrs)	1.0	0.2	0.8	0.4	1.3	0.2	1.4	0.3
AUC (0–24)	(ng·hr·ml ⁻¹)	2.0	1.1	0.7	0.3	19.5	6.1	14.3	5.7

et al. 1982), but this cannot be supported by *in vitro* studies (Corell *et al.* 1983). A third possibility may be indirect effects on thromboxane synthesis, possibly via other arachidonic acid metabolites.

For each prostanoid the concentration response relationships after the two administrations were similar (fig. 5). It is thus likely that gastrointestinal and liver passage does not alter the activity of fenflumizole.

In conclusion the pharmacokinetics of fenflumizole given intravenously to healthy subjects followed a three compartment model with plasma half-lives of 2 min., one and 15 hours. The two

last half-lives agreed with those observed following oral administration of the drug.

The volume of distribution of the peripheral compartment was 386 l, indicating tissue distribution. A slow elimination from tissues following repeated dosage could not be excluded.

The clearance from plasma amounted to 0.5 l per min. and was suggested to reflect mainly metabolic activity and biliary or intestinal excretion. The high hepatic clearance resulted in a pronounced first-pass effect, reducing the oral availability of fenflumizole to fifty percent.

A rapid absorption of oral fenflumizole was

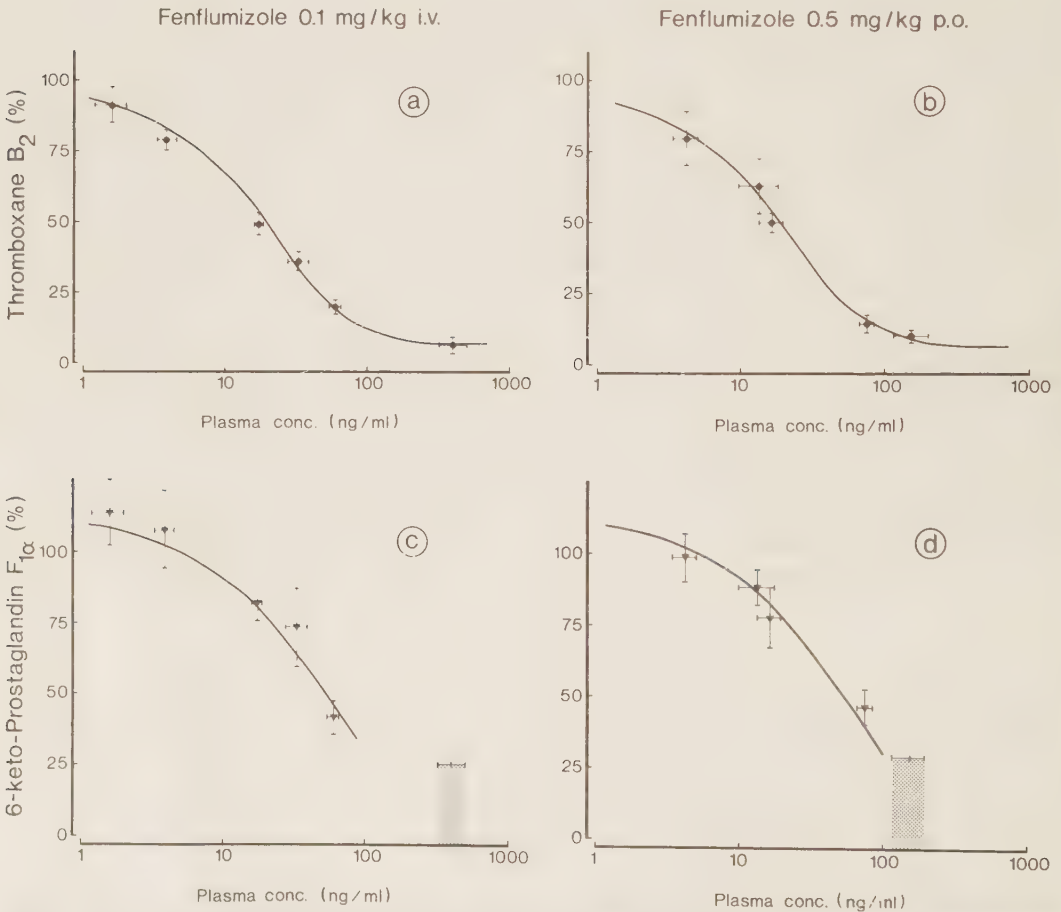


Fig. 5. Immunoreactive thromboxane B₂ (a + b) and 6-keto-prostaglandin F_{1α} (c + d) in serum as % of predose-levels, against concomitant plasma concentrations of fenflumizole after intravenous (a + c) and oral (b + d) doses to volunteers (mean values $\pm$ S.E.M., n = 8). Shaded areas represent 6-keto-PGF_{1α}-levels below the detection limit of the assay.

demonstrated rendering peak plasma concentrations in one hour. Compared with earlier data from the same subjects the bioavailability was constant over the dose-range 0.1–2 mg per kg. The two metabolites, mono- and didemethyl fenflumizole were detected in low concentrations after both routes of administration, showing maximum plasma concentrations at 1–2 hours.

Fenflumizole inhibited formation of immunoreactive TXB₂ as well as 6-keto-PGF_{1α}, and appeared to be two- to threefold more potent on TXB₂, which confirms previous findings.

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PHARMACOKINETICS OF ^{14}C -FENFLUMIZOLE
AFTER INTRAVENOUS ADMINISTRATION TO MAN

by

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Running title: Intravenous ^{14}C -fenflumizole to man.

ABSTRACT

The disposition of ^{14}C -labelled fenflumizole was studied in five healthy subjects who received 0.1 mg/kg of fenflumizole as single intravenous doses. Radioactivity was measured in whole blood, plasma, urine and faeces from prior to until 168 hours after dose administration. Chemical determinations of fenflumizole and its two demethylated derivatives in plasma were made by HPLC followed by fluorescence detection. Concentrations of fenflumizole calculated from radioactivity in plasma were consistently higher than from chemical determinations, suggesting the presence of metabolites. Radioactivity in blood cells decreased more slowly than in plasma. Plasma concentrations versus time could be described by a four-compartment model, with a terminal half-life of 119 hours (median). About 50 % of the dose was excreted with the faeces, and about 4 % was recovered in the urine. Unchanged fenflumizole in the urine accounted for 0.0001 % of the total dose. Renal clearance of radioactive material decreased at low concentrations of radioactive compound in plasma, which may indicate changes in unbound fraction of drug and metabolites, or a saturable tubular reabsorption process. The slow elimination of fenflumizole is consistent with slow release from tissues and/or enterohepatic recycling.

Key words: fenflumizole - pharmacokinetics - intravenous dosing - anti-inflammatory drug - healthy subjects

INTRODUCTION

Fenflumizole (2-2(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)-imidazole is a recently developed compound with anti-inflammatory and analgesic properties in animal models (Corell & Hasselmann 1983). It is a potent inhibitor of formation of prostanoids in vitro and in vivo animals (Corell et al. 1983) and in humans (Vinge et al. 1984, 1985). In contrast, its gastro-ulcerogenicity and its acute toxicity in animals appears to be reduced in comparison with other potent inhibitors on prostanoid generation (Corell & Hasselmann 1983).

The pharmacokinetics of fenflumizole in man have previously been described after single oral and intravenous doses (Midskov et al. 1984, Vinge et al. 1985). In spite of a bioavailability of 49 % only very small amounts of oral doses (less than 1 %) could be recovered in the urine during the first 24 hours. Nevertheless, the clearance from plasma was high (0.54 l/min), which suggested that fenflumizole is extensively metabolized or excreted in the bile or otherwise into the intestine. In rats and dogs, studies with radiolabelled fenflumizole have shown a high degree of fecal excretion (Midskov & Arnold, unpublished results).

The aim of the present study was to obtain further information on the human patterns of metabolism and excretion of fenflumizole. For this purpose ¹⁴C-labelled drug was administered to volunteers in single intravenous doses.

MATERIALS AND METHODS

Subjects

Five healthy males volunteered in the study. The protocol had been approved by the Ethics Committee of the University of Lund, Sweden, and informed consent was obtained from each subject. None of them had received any other radiolabelled compound during the year preceding the study. Their ages ranged from 26-35 years (median 33 years) and their body weights between 65 and 86 kg (median 73 kg). Laboratory tests including hematocrit and counts of blood cells, transaminases and serum creatinine were normal.

Drug

Fenflumizole (U.S. patent 5219) was labelled in the 2-position of the imidazole nucleus (Figure 1). The radioactive purity was in excess of 99 % (Scott & Hawkins 1982). A solution for intravenous infusion was made up with Carbowax 300, propyleneglycol and hydrochloric acid, in the same proportions as used previously for unlabelled drug (Vinge et al. 1985).

Procedures

^{14}C -Fenflumizole 0.1 mg/kg was administered as an infusion over 5 minutes into an antecubital vein. The first subject, who was regarded as a pilot, received a radioactive dose of 0.34 $\mu\text{Ci/kg}$ (total dose 22 μCi), and the other four subjects received 1.39 $\mu\text{Ci/kg}$ (range of total doses 95-120 μCi). The dose was always given at 7.30-8.00 a.m. The subjects had been fasting overnight and remained so until 3 hours after the dose, at which time a standard breakfast was served.

Venous blood was collected in heparinized tubes prior to (= -5 min), at the end of (= 0 hours) and at 5, 15 and 30 minutes, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after the infusion. Duplicate samples of 200 μl of the blood were immediately taken and allowed to dry in porous paper caps. The remaining blood was allowed to stand in room temperature for 30

minutes before plasma was separated and immediately frozen at -20°C .

Urine was collected in opaque plastic bottles during the time intervals 0-2 hours, 2-4, 4-8, 8-12, 12-36, 36-60, 60-84, 84-108, 108-132, 132-156 and 156-168 hours. The portions were weighed and samples were stored at -20°C until analysis.

All faeces was collected as portions and stored like the urine pending radioactivity counting.

The solutions containing the drug and the collected samples were always protected from natural light, due to the photolability of fenflumizole (Midskov, unpublished). Laboratory work was carried out in subdued lighting or gold fluorescent light.

Analysis of fenflumizole in plasma and urine

Determination of fenflumizole and its mono- and di-demethylated derivatives (hereafter called MD-FFZ and DD-FFZ) was performed as described previously (Midskov 1983). In addition, the urine assay could quantify the sulfate and glucuronic acid conjugates of MD-FFZ and DD-FFZ. In brief, each sample of urine was divided into three portions for separate liquid chromatography analysis of 1) fenflumizole, MD-FFZ and DD-FFZ, 2) glucuronide conjugates of the metabolites and 3) sulfate conjugates of the metabolites. Following purification and extraction the samples were either chromatographed without further processing (fenflumizole, MD-FFZ, DD-FFZ) or subjected to enzymatic release (conjugated metabolites) prior to chromatography. Two different eluents were employed in order to comply with the polarity differences between the parent compound and its metabolites. A reversed-phase column was used and the compounds eluted isocratically at a temperature of $50-55^{\circ}\text{C}$. Details of the urine assay will be published in a forthcoming paper.

The lower limit of determination for the compounds quoted was 100-200 pg per 1 ml of plasma or 5 ml of urine. Precisions at the concentrations studied amounted to relative standard deviations of

5-7 %.

Measurement of radioactivity in plasma, blood, urine and faeces

^{14}C -radioactivity was counted with a Beckman Liquid Scintillation 150 Counter. Quench correction was made by the method of external standards. Radioactivity in plasma and urine was measured in crude samples, whereas faeces and blood were first combusted in an oxidizer (B 306 Tri Carb, Packard Instruments, USA). Limits of determination were below 1 ng/ml in all cases. Details on the procedures will be published elsewhere.

Data processing

Symbols and abbreviations used in the text are presented in Table 1.

Sample concentrations were determined using conventional linear calibration curves based on biological samples to which were added fenflumizole and the two metabolites.

The course of fenflumizole in plasma versus time was described by a four-compartment open model expressed by the following equation:

$$C_p = C_1 \cdot \exp(-\lambda_1 t) + C_2 \cdot \exp(-\lambda_2 t) + C_3 \cdot \exp(-\lambda_3 t) + C_z \cdot \exp(-\lambda_z t)$$

The fitting procedures were performed by means of the method of simplex (Yamaoka et al. 1981) combined with a modified Marquardt method (Nielsen & Houbak, personal communication).

Areas under the plasma concentration-time curve (AUCs) were determined using the trapezoidal rule until the last observed concentration. AUC from 168 hours to infinity was then calculated by dividing the last observed concentration by the individual terminal elimination rate constant λ_z . Model based AUCs were calculated as

$$\text{AUC} = \frac{C_1}{\lambda_1} + \frac{C_2}{\lambda_2} + \frac{C_3}{\lambda_3} + \frac{C_z}{\lambda_z}$$

Plasma clearance (CL_p) and renal clearance (CL_R) were conceived as

$$CL_p = \frac{\text{Dose}}{AUC} \quad \text{and} \quad CL_R = \frac{A_e(\infty)}{AUC}$$

Apparent volume of the central compartment (V_c) and apparent volume of distribution at steady state (V_{ss}) were conceived as (ACCP report 1982)

$$V_c = \frac{\text{Dose}}{C_1 + C_2 + C_3 + C_z}$$

and

$$V_{ss} = \text{Dose} \times \frac{C_1/\lambda_1^2 + C_2/\lambda_2^2 + C_3/\lambda_3^2 + C_z/\lambda_z^2}{AUC^2}$$

The relationship between blood and plasma radioactivity was evaluated for each subject by means of the ratio of radioactivity in blood (R_b) to that in plasma (R_p) according to the equation

$$R_b = R_p(1 - \text{Hct}) + R_e \cdot \text{Hct},$$

where R_e denotes radioactivity in erythrocytes and Hct haematocrit.

RESULTS

Concentrations of fenflumizole and radioactivity in plasma and blood

Median levels of the plasma concentrations of fenflumizole and radioactivity in blood and plasma, expressed as equivalents of fenflumizole, are shown in Figure 2. The maximum levels after the end of the infusion ranged between 67 and 292 ng/ml for fenflumizole determined chemically, and between 77 and 363 ng/ml as calculated from radioactivity in plasma. When measured in whole blood the values found at 0 hours ranged from 86 to 395 ng/ml. In plasma, as well as in blood, concentrations showed an initial rapid decline over the first five minutes followed by an intermediate phase and after about 12 hours a slow terminal phase appeared. At 24 hours the concentration of fenflumizole in plasma was 0.7-1.5 ng/ml, of ^{14}C -activity 2.4-5.5 ng/ml and of ^{14}C -activity in whole blood 5.4-16.3 ng/ml. The two latter terms are expressed as equivalents of fenflumizole. Calculated pharmacokinetic parameters for a four-compartment model describing the plasma profile of fenflumizole are presented in Table 2. The two radioactivity profiles were not subjected to compartmental analysis, but it was found that the terminal elimination phase of radioactivity in plasma declined at approximately the same rate as fenflumizole in plasma. As is visualized in Figure 2, the curve of radioactivity in whole blood versus time flattened out, with an even slower rate of disappearance than observed in plasma.

Individual curves of concentrations in plasma and blood contain at 8-10 hours an anomaly in the smooth concentration decline. This short plateau, or hump, was a consistent finding, although more or less pronounced (Figure 3).

The partition ratio of radioactivity in blood cells and plasma versus time is illustrated in Figure 4 as a median curve. Although the absolute amounts of radioactive substance in the blood cells continuously decreased, the relative amount increased over the entire observation period. Partition equilibrium occurred at 1-1.5 hours after dose administration.

Excretion in the urine

Median values for cumulated radioactivity in the urine are presented in Figure 5 as per cent of the dose of ^{14}C -fenflumizole. Radioactive material corresponding to 3.8% of the dose was excreted by the kidneys and only one fourth hereof has been identified until now. In all, 0.0019% of the dose was renally excreted fenflumizole and unconjugated metabolites. Unchanged fenflumizole accounted for 0.0001% (median value) and was recovered in the first 8 hours. Of the identified compounds, the major part was made up by the glucuronide of MD-FFZ (Table 3).

The rate of excretion of radioactivity (scaled as % of dose per hour) was plotted against concentrations of radioactivity in plasma (measured in equivalents of fenflumizole as ng/ml) at mid-time of corresponding urine collection intervals (Figure 6). Data from the first urine collection period were left out in the plot, because of the probable delay in the urinary excretion during that period. The renal clearance plot revealed that clearance (equal to slope) of radioactive substance decreased at low concentrations in plasma.

The cumulative recovery of labelled compound in the urine was about 4% over one week, which is very low. Unchanged fenflumizole accounted for 0.0001% (median) and was recovered in the first 8 hours. About three fourth of the total activity in urine represents not yet identified material.

Excretion of radioactivity in the faeces

Cumulated excretions ^{14}C -activity in the faeces are presented in Figure 7. It appears that about half of the dose was recovered within a collection period of 168 hours. For 4 of the subjects the course of faecal excretion had not yet formed an upper plateau from which the ultimate amount for excretion can be extrapolated.

DISCUSSION

The concentrations in plasma of intravenously administered ^{14}C -fenflumizole over 24 hours agree well with the results obtained with unlabelled drug at the same dosage (Vinge et al. 1985). In the present study, however, the observation period was extended to one week and as the assay of fenflumizole allows determination of low drug-levels, we have now been able to demonstrate a terminal elimination rate of fenflumizole, which is much slower than previously suggested. The terminal plasma half-lives seen in this study reach figures in the order of 50-190 hours.

In the plasma concentration-time curves there was a tendency towards formation of a plateau, or hump, at 10-12 hours after dose administration. Actually, this was already noticed in our foregoing study with unlabelled drug (Vinge et al. 1985), but was not discussed. However, since the present study confirms this early observation (Figure 3), we now suggest that this as an indication of enterohepatic recycling of fenflumizole, which also would be in concert with a slow elimination rate and a high degree of faecal excretion. Enterohepatic recirculation was previously suggested for other non-steroid antiinflammatory drugs like indomethacin (see Helleberg 1981), flufenamic acid and piroxicam (see Verbeeck et al. 1983).

The concentration of labelled compound in plasma was higher than the concentration of chemically determined fenflumizole. This result could suggest impurities in the ^{14}C -fenflumizole solution, which however was excluded in repeated control experiments.

Since the two hitherto identified metabolites MD-FFZ and DD-FFZ, cannot account for the discrepancy between the concentrations of radioactive compound and chemically determined fenflumizole it follows that additional metabolites are present in plasma.

Radioactivity in blood was lower than in plasma during the first hour, but the concentration ratio in blood cells versus plasma then gradually increased to about 20:1 at 168 hours (Figure 4). This finding points at drug binding to or uptake by the blood

cells, which eventually release the drug extremely slowly with a rate as reflected by the terminal elimination phase of radioactivity in whole blood. The blood cells thus appear to act as a deep compartment. At 168 hours about 0.4% of dose (about 30 µg) remained in the blood. As only little changes in absolute amounts of radioactivity in whole blood were assessable between 24 and 168 hours it implies that practically irreversible binding takes place.

The cumulated recovery of labelled substance in the urine was unusually low for antiinflammatory drugs (Verbeeck et al. 1983). The maximum rate of excretion of unchanged fenflumizole found in any subject was 0.0001% of the administered dose over 2 hours, which corresponds to a clearance rate of about 0.14 ml/h or 0.002 ml/min. Such a low renal clearance would suggest filtration of a drug, that is 99.998% bound to plasma proteins or that the drug is reabsorbed from the tubuli (Rowland & Tozer 1980). Urine pH will favour extensive passive reabsorption of very weakly basic nonpolar drugs, such as propoxyphene (Rowland & Tozer 1980) and fenflumizole. In addition the plot of excretion rate of labelled compound (% of dose per hour) versus mid-time concentrations in plasma (measured as radioactivity in equivalents of fenflumizole) showed that renal clearance was not constant but decreased at low plasma concentrations. This may indicate that the fraction unbound to plasma proteins increases with increase of the total concentration of drug, as was also shown for naproxen (Runkel et al. 1974). If the fraction unbound is constant, however, the result would be consistent with an active reabsorption process for components of or the sum of labelled material (Rowland & Tozer 1980). Both passive reabsorption, i.e. non-ionic diffusion, and carrier-mediated reabsorption, as well as some degree of tubular secretion appears to exist for salicylate (Møller & Sheikh 1983). Passive reabsorption probably also occurs with phenylbutazone under normal conditions, whereas during alkalosis tubular secretion predominates (Møller & Sheikh 1983). Active tubular secretion is believed to occur normally with indomethacin (Helleberg 1981), and with sulfinpyrazone (Møller & Sheikh, 1983).

Recovery of radioactivity in the faeces shows that this is the major route of excretion of fenflumizole and its transformation products. However, still after 168 hours not all individual curves of cumulated excretion had flattened out indicating that there was some amounts yet to be excreted by this route. As discussed above, a high degree of excretion into the intestine would favour entero-hepatic recycling of the drug.

The present study thus presents data that are suggestive of processes which cannot be described by linear pharmacokinetic models. Although there was a linear relationship between doses and AUCs in our previous studies (Midskov et al. 1984, Vinge et al. 1985) calculations did not take into account plasma levels of fenflumizole below 1 ng/ml, where the slow terminal elimination phase can be discerned. At present, however, we do not know how this phase contributes to the kinetics at higher and/or repeated doses. Also, it remains to be investigated whether pharmacological or toxicological effects can be related to the slow elimination, or to the penetration per se of the drug into tissues which act as deep compartments.

In summary, the present results confirm previous findings that renal elimination of fenflumizole accounts for a very small part of the total clearance. The main part of the administered intravenous doses of ^{14}C -labelled fenflumizole was eliminated through the intestinal tract, possibly via excretion into the bile. However, after 7 days the metabolic fate of about 40% of the given dose was not clarified. In view of the high apparent volume of distribution for fenflumizole, it is possible that the drug is tightly bound to tissues and hence is released very slowly from these binding sites. Other possible explanations for the unknown 40% could theoretically be that the drug is excreted through skin glandules or, more speculatively, that a complete break-down of fenflumizole leads to formation of $^{14}\text{CO}_2$ which leaves the body with the breath.

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Table 1: Symbolism for pharmacokinetic parameters and compounds.

Symbol	Definition	Unit
t	Time	h
C_p	Concentration of fenflumizole in plasma at any time t	ng/ml
$t_{\max}$	Time to reach $C_{\max}$	h
$C_{\max}$	Maximum plasma concentration	ng/ml
C_1	Zero time intercept of the λ_1 -line	ng/ml
C_2	Zero time intercept of the λ_2 -line	ng/ml
C_3	Zero time intercept of the λ_3 -line	ng/ml
C_Z	Zero time intercept of the λ_Z -line	ng/ml
λ_1 - λ_Z	Rate constants associated with distribution and elimination	h^{-1}
$t_{\frac{1}{2}\lambda_1}$ - $t_{\frac{1}{2}\lambda_2}$	Half-lives corresponding to λ_1 - λ_Z	h
R_p	Radioactivity in plasma at any time t	*
R_b	Radioactivity in whole blood at any time t	*
R_e	Radioactivity in erythrocytes at any time t	*
V_c	Pharmacokinetic volume of central compartment	l
V_{ss}	Pharmacokinetic overall volume at steady state	l
AUC	Area under the concentration-time curve from zero to infinity	$\text{ng}\cdot\text{h}\cdot\text{ml}^{-1}$
CL_p	Total body clearance from the body	ml/h
CL_R	Renal clearance from plasma	ml/h
$A_e(0-t)$	Amount of intact fenflumizole recovered in urine until time t	ng
MD-FFZ	Mono demethyl fenflumizole	-
DD-FFZ	Di demethyl fenflumizole	-
Glu-/Sulf-	Prefixes denoting conjugates with glucuronic acid or sulfate	-
Hct	Haematocrit	-

* Measured in equivalents of fenflumizole as ng/ml

Running title: Intravenous ^{14}C -fenflumizole to man.
 Authors: Vinge E., C. Midskov and E. Arnold.

Table 2: Pharmacokinetic fenflumizole (0.1 mg/kg) parameters of five healthy males inferred from computer aided fitting of measured plasma concentrations to a four-compartment open model.
 Units as denoted in table 1.

Parameter	Subject no.					Median
	1	2	3	4	5	
C_1	242	231	259	28.6	33.3	231
λ_1	16.5	24.7	36.0	2.06	116	24.7
$t_{\frac{1}{2}\lambda_1}$	0.04	0.03	0.02	0.34	0.006	0.03
C_2	44.1	45.0	39.9	33.9	31.4	39.9
λ_2	0.57	0.42	4.5	0.57	0.57	0.57
$t_{\frac{1}{2}\lambda_2}$	1.22	1.65	0.15	1.22	1.22	1.22
C_3	3.35	2.19	19.8	3.65	1.95	3.35
λ_3	0.06	0.05	0.39	0.084	0.042	0.06
$t_{\frac{1}{2}\lambda_3}$	11.5	13.9	1.78	8.25	16.5	11.5
C_z	0.73	0.59	0.86	0.49	0.34	0.59
λ_z	0.0099	0.0036	0.014	0.0045	0.0058	0.01
$t_{\frac{1}{2}}$	70	192	49	154	119	119
$\text{AUC}_{\text{model}}$	222	324	128	226	160	222
CL_p	33033	22531	61719	30088	53750	33033
CL_R	498	648	1000	740	1130	740
V_c	25.2	26.3	24.7	10.2	12.9	24.7
V_{ss}	1261	3245	2179	3305	3799	3245

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Table 3: Composition of faecal and renal excreta following intravenous injection of ^{14}C -labelled fenflumizole to five healthy males.

Compound	Excretion route	Amount excreted (% of dose)	
		Range	Median
^{14}C -activity	Faecal	67.2 - 47.3	50.5
^{14}C -activity	Renal	4.4 - 3.1	3.8
Glu-MD-FFZ	Renal	1.1 - 0.25	0.7
Glu-DD-FFZ	Renal	0.08 - 0.003	0.04
Sulf-MD-FFZ	Renal	0.07 - 0.004	0.04
Sulf-DD-FFZ	Renal	0.002- 0.001	0.001
Fenflumizole	Renal	0.0001	0.0001
MD-FFZ	Renal	0.002-0.0003	0.0008
DD-FFZ	Renal	0.003-0.0003	0.001

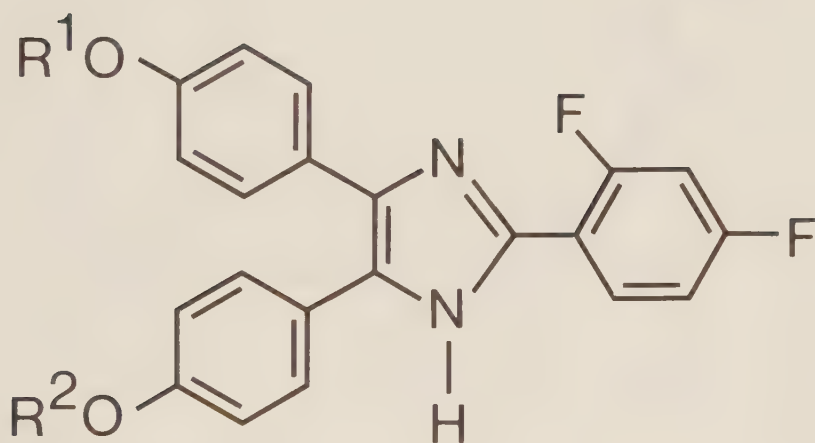


Figure 1:

Molecular structures of fenflumizole and known metabolites.

Fenflumizole: R¹ and R² equal to CH₃. MD-FFZ: Either R¹ or R² replaced by H; DD-FFZ: Both R¹ and R² replaced by H. Conjugation with glucuronic acid or sulfate takes place at R¹ and/or R².

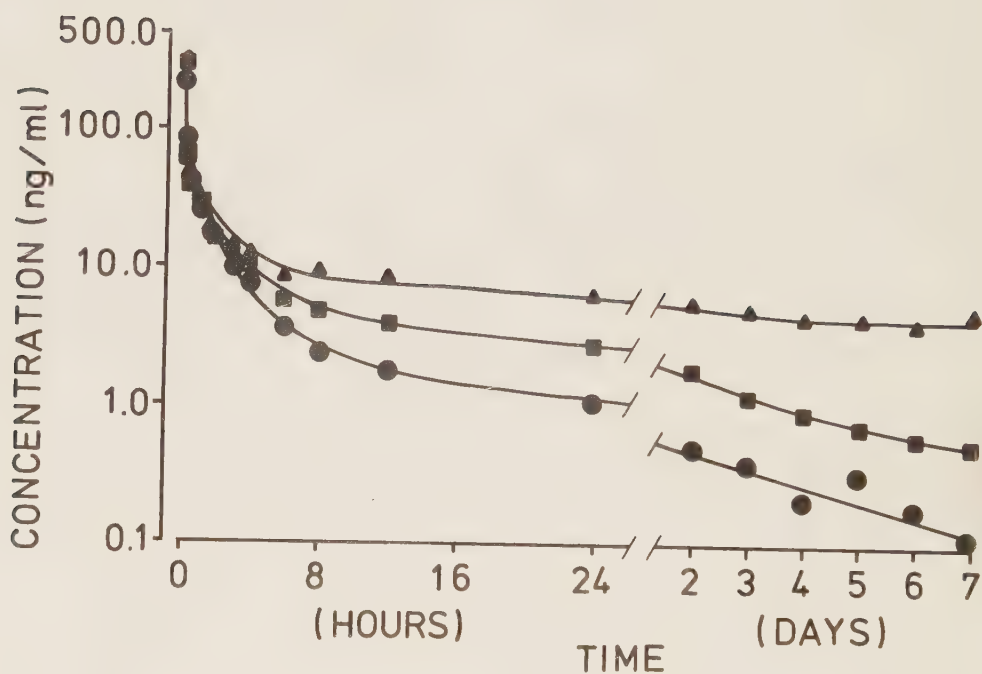


Figure 2:

Median plasma concentrations following intravenous injection of 0.1 mg/kg of ^{14}C -fenflumizole to five male volunteers: radioactivity in whole blood $\blacktriangle$ (upper curve), radioactivity in plasma $\blacksquare$ (middle), fenflumizole in plasma $\bullet$ (lower).

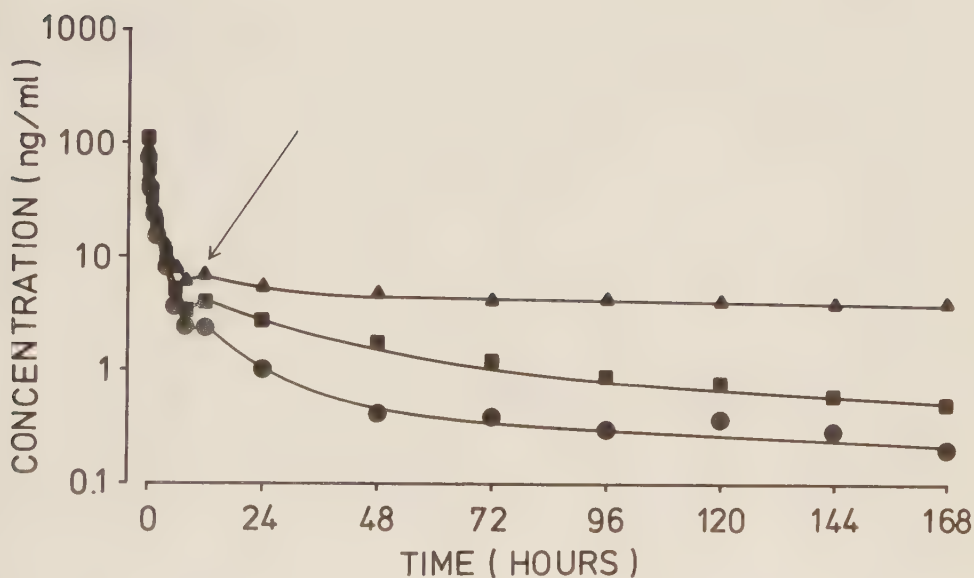


Figure 3:

Plasma concentrations following intravenous injection of 0.1 mg/kg of ^{14}C -fenflumizole to subject no. 4: radioactivity in whole blood $\blacktriangle$ (upper curve), radioactivity in plasma $\blacksquare$ (middle), fenflumizole in plasma $\bullet$ (lower). Note plateau formation at arrow.

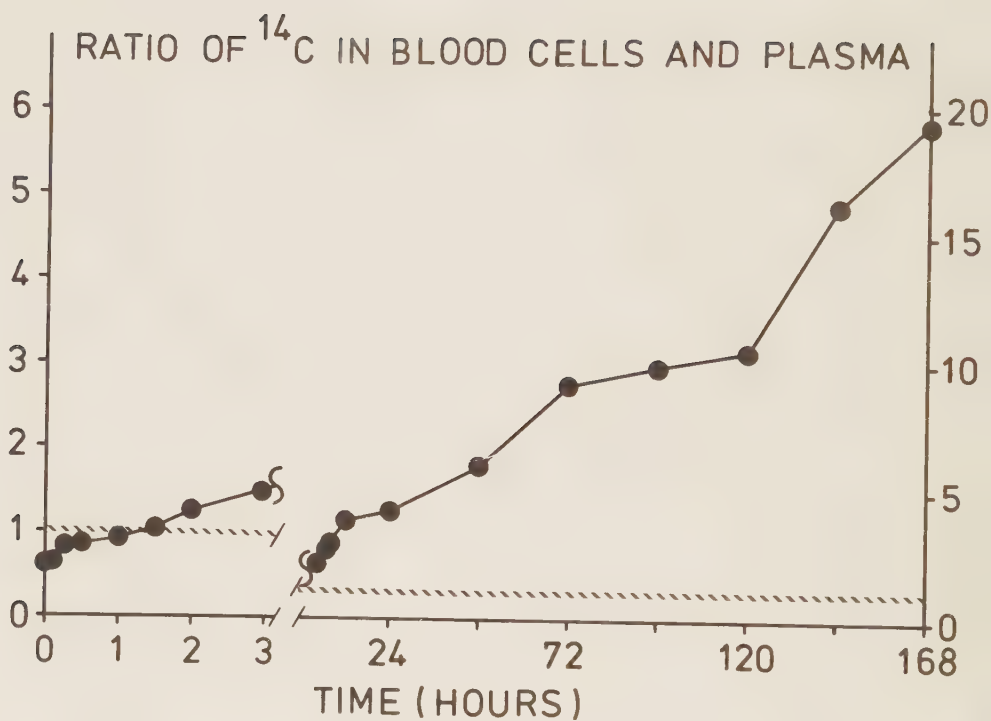


Figure 4:

In vivo partition of radioactivity to blood cells and plasma following an intravenous injection of 0.1mg/kg of ^{14}C -labelled fenflumizole to male volunteers (median of five). The dotted lines indicate partition equilibration in the system before and after 3 hours.

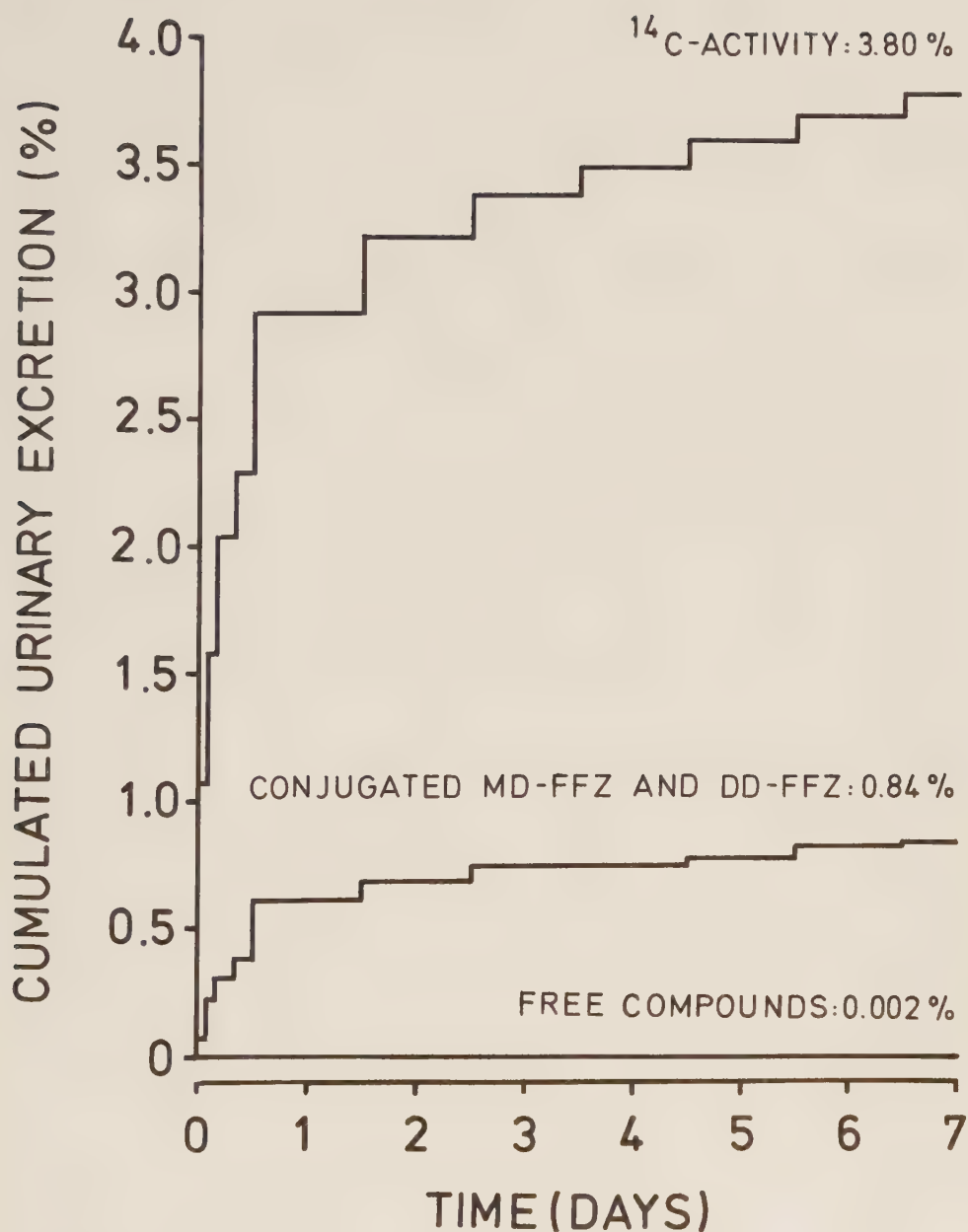


Figure 5:

Cumulated urinary excretions in per cent of dose following intravenous injection of 0.1 mg/kg of ^{14}C -labelled fenflumizole to male volunteers (median of five).

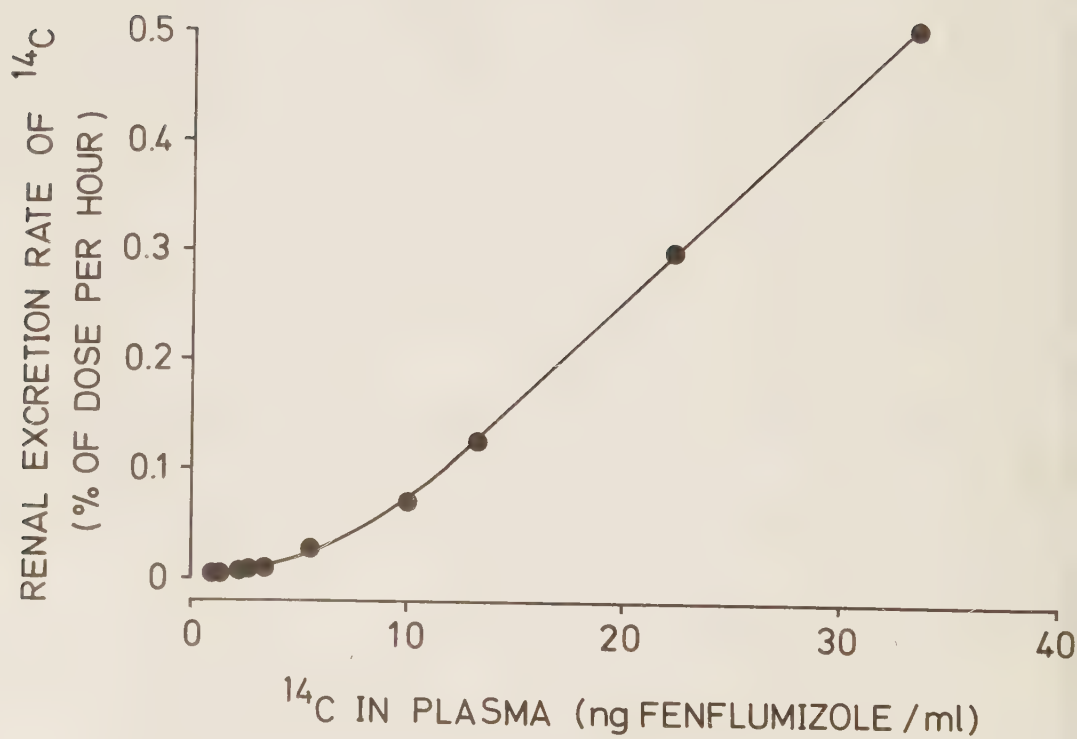


Figure 6:

The relationship between the rate of renal excretion and plasma concentration, both measured as radioactivity, for subject no. 1.

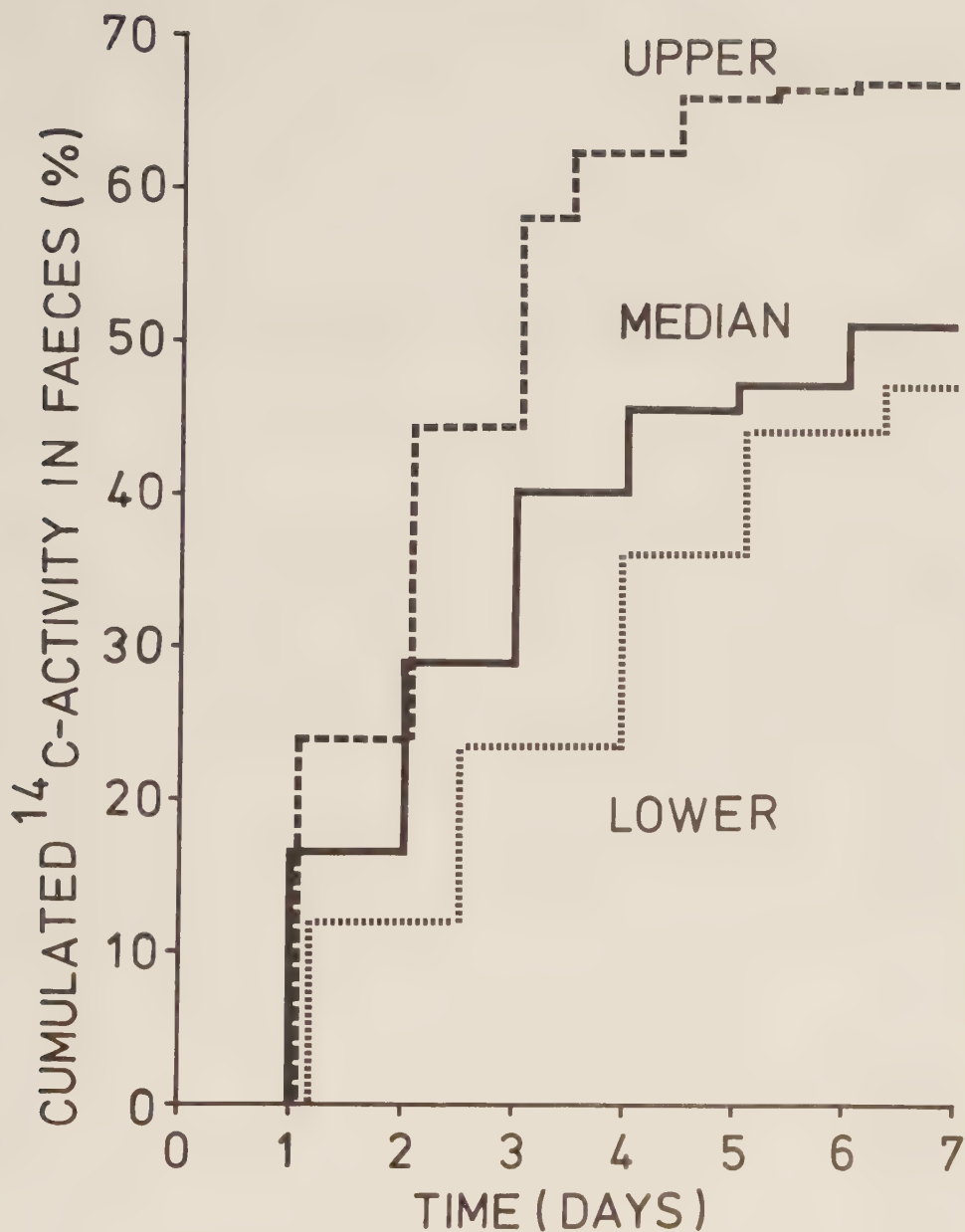


Figure 7:

Cumulative faecal excretions in per cent of dose following intravenous injection of 0.1 mg/kg of ^{14}C -labelled fenflumizole to five male volunteers. Measured maximum, minimum and median excretion courses are shown.

Effects of Fenflumizole on Aggregation Ex Vivo of Human Platelets and Formation of Thromboxane B_2 and 6-Keto-Prostaglandin- $F_{1\alpha}$

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Summary. Fenflumizole (2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole), a new non-steroidal anti-inflammatory drug, was given to healthy subjects in single oral doses of 0.1, 1 and 2 mg/kg. The effect of the drug was followed for up to 8 h by repeated tests of arachidonic acid-induced platelet aggregation and was related to its concomitant plasma concentration. Fenflumizole reversibly inhibited platelet aggregation and the degree of inhibition was found to be linearly correlated with the log plasma concentration. There was depression of the formation of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ (the stable metabolites of thromboxane A_2 and prostacyclin) in clotted whole blood measured by radioimmunoassay after fenflumizole 1 mg/kg. This effect was directly related to the concentration of the drug in plasma, the maximum effect being reached at fenflumizole concentrations > 200 ng/ml. EC_{50} -values for inhibition of the formation of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were approximately 20 and 40 ng/ml, respectively. The results suggest that orally administered fenflumizole is a potent inhibitor of platelet aggregation and prostanoid formation.

Key words: fenflumizole, thromboxane formation, prostacyclin formation; platelet aggregation, arachidonic acid, healthy subjects, blood clotting

troulcerogenicity in laboratory rodents [1], and furthermore, the acute toxicity of fenflumizole was extremely low in those species ($LD_{50} > 2000$ mg/kg); [1]. Fenflumizole is a potent cyclooxygenase inhibitor in vitro, ex vivo and in vivo, with activities comparable to those of indomethacin [2]. The drug exerts antiplatelet properties, i.e. it is a strong inhibitor of platelet aggregation in vitro and in vivo [2]. Because of this pharmacodynamic profile it was decided to investigate the effects of fenflumizole in human volunteers on platelet aggregation induced by arachidonic acid. In addition, the plasma concentrations of fenflumizole were estimated, and the formation in clotting whole blood of thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$), the stable metabolites of thromboxane A_2 (TXA_2) and prostacyclin (PGI $_2$), were measured after fenflumizole 1 mg/kg.

Materials and Methods

Eight healthy males gave their written informed consent to participation in the study. Their ages ranged from 24 to 41 years (mean 30 years) and their body weights from 62 to 83 kg (mean 71 kg). Before entering the study their health was checked by physical

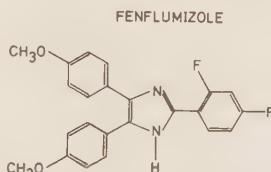


Fig. 1. Structural formula of fenflumizole. Molecular weight 392.39

Fenflumizole is a new non-steroidal anti-inflammatory imidazole derivative. In experimental animals it has been shown to possess anti-inflammatory, analgesic and anti-pyretic activities similar to those of indomethacin. However, fenflumizole had markedly fewer side-effects than indomethacin, such as gas-

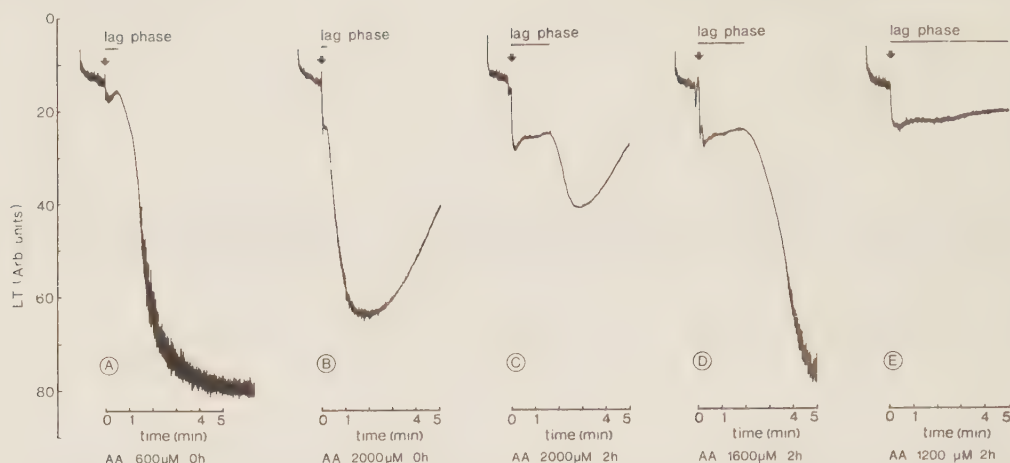


Fig. 2 A-E. Arachidonic acid(AA)-induced platelet aggregation in platelet rich plasma. Arrows indicate addition of AA giving final concentrations as indicated below the tracings. LT = light transmission in arbitrary units. **A** and **B** in drug-free plasma (0 h), **C-E** 2 h after fenflumizole 1 mg/kg. The length of the lag phase is indicated above the tracing

Table 1. Antiaggregatory activity of fenflumizole in vitro and plasma concentrations of the drug after oral administration to human volunteers. Platelets were aggregated with arachidonic acid (AA). AA_{1 min} = AA-concentration (µM) inducing aggregation in 1 min

Dose of fenflumizole mg/kg	Time after dose h	AA _{1 min} (µM)		Plasma fenflumizole (ng/ml)	
		mean ± SEM	n	mean ± SEM	n
0.1	0	480 ± 43	7	0 ± 0	8
	½	1490 ± 173	7	19.5 ± 5.5	8
	1	1760 ± 82	7	29.5 ± 7.6	8
	2	1700 ± 234	6	18.2 ± 2.8	8
	3	1460 ± 190	6	11.4 ± 2.0	8
	4	1170 ± 136	5	7.4 ± 1.4	8
	6	1060 ± 135	5	4.4 ± 1.1	8
	8	860	3	3.0 ± 0.8	8
1	0	522 ± 32	6	0 ± 0	8
	½	2180 ± 195	6	157 ± 42.6	8
	1	2200 ± 195	6	199 ± 40.0	8
	2	1960 ± 79	5	161 ± 24.5	8
	3	1720	2	127 ± 21.3	8
	4	2200 ± 234	6	82 ± 13.2	8
	6	2060 ± 151	6	44.4 ± 6.3	8
	8	2130 ± 232	4	33.3 ± 6.6	8
2	0	486 ± 41	7	0 ± 0	8
	½	1930 ± 92	7	168 ± 50.7	8
	1	1890 ± 94	7	352 ± 72.6	8
	2	1820 ± 96	4	272 ± 49.4	8
	3	2050	2	214 ± 37.6	8
	4	2120 ± 121	6	151 ± 28.0	8
	6	1980 ± 113	7	94 ± 17.1	8
	8	1920 ± 54	6	60 ± 11.3	8

examination, medical history, ECG and laboratory tests. The study was approved by the Ethics Committee of the University of Lund, Sweden.

Fenflumizole (2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl) imidazole; Fig. 1), synthesized by Dumex Ltd., was given in single oral doses prepared as an aqueous suspension in Tween 80, tragacanth, sorbic acid and ethanol (final concentrations of 0.1%, 0.375%, 0.05% and 0.375%, respectively). The fenflumizole concentration was 0.5 or 5 mg/ml.

Fenflumizole was given at 3 different dose-levels, 0.1, 1 and 2 mg/kg in volumes of 0.2 or 0.4 ml/kg. Each volunteer received all 3 doses at intervals of at least 7 days. They had not taken any anti-inflammatory drugs during the week preceding each dose, except once when the subject forgot and took a single dose of aspirin 4 days before the trial day. In another series of experiments, the 1 mg/kg dose was repeated for measurements of TXB₂ and 6-keto-PGF_{1α}-formation.

The subjects fasted from midnight before each trial day. No food or beverages were allowed until 3 h after the dose, when a standardized meal was served. After 4 h there was no restriction on the diet, except for alcohol, which was prohibited through the trial day.

Blood pressure and heart rate were monitored during the first 4 h after the dose, and the subjects were carefully observed for adverse reactions throughout the study.

Platelet Aggregation

Platelet aggregation tests were performed prior to (zero sample) and 0.5, 1, 2, 3, 4, 6 and 8 h after medication. Blood was taken from an antecubital vein through a short indwelling catheter (Venflon®) and stabilized with sodium citrate (final concentration 0.38%). Platelet rich plasma (PRP) was obtained by centrifugation (190 g, 10 min) and collection of the supernatant. Platelet poor plasma (PPP) was prepared by further centrifugation of the cell fraction (2000 g, 15 min). Arachidonic acid (AA; Nu-Chek-Prep, Elysian, MN, USA) was used as the proaggregatory substance. Platelet aggregation was measured in an HU Aggregometer (37 °C, stirring at 1100 r.p.m.) connected to a Vitatron Recorder. The principle is an increase in light transmission in parallel with

aggregation in PRP placed in a siliconized cuvette in the aggregometer [3]. The % light transmission was recorded with preset values of approximately 10 for PRP and approximately 90 for PPP. AA was added as the sodium salt (50 mg AA was dissolved in 1.4 ml ethanol plus 0.2% Na₂CO₃ 6.1 ml; 10 µl of this solution in 1 ml of PRP equals approximately 200 µM AA). The aggregatory response to varying concentrations of AA in PRP was tested, ranging from concentrations causing no aggregation within 5 min to concentrations of AA which triggered aggregation within 1 min. The time from addition of AA to the beginning of aggregation was recorded (T_{agg}); see Fig. 2.

Aggregation was considered to have begun when the initial decrease in light transmission, which is due to a change in shape of the platelets [4, 5], changed into a clear increase. This phase in the aggregation curve could be identified in most of the recorded curves and preceded both irreversible and reversible aggregation. For each sampling time the AA-concentration that initiated aggregation in 1 min (AA_{1min}) was calculated by relating the AA-concentrations used to the measured aggregation times. Administration of vehicle only to two subjects did not affect the AA_{1min}-values over a 6 h observation period.

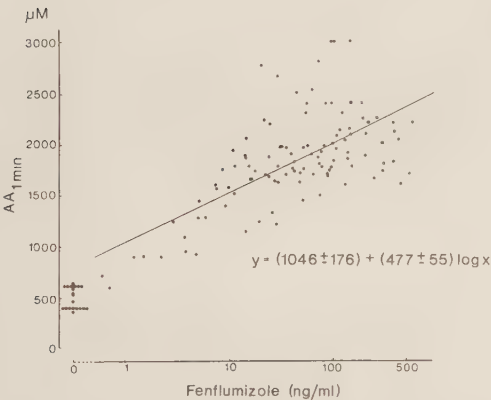


Fig. 3. Concentrations of AA required to start aggregation of platelets in 1 min (AA_{1min}) plotted against plasma fenflumizole concentration. Individual values and mean ± SEM of the regression lines (n = 7) are given

Drug Concentration in Plasma

Plasma concentrations of fenflumizole were followed through 24 h after each dose. Blood was collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after the dose. When the 1 mg/kg dose was repeated blood was collected at 0, 1, 6 and 24 h. Blood was collected in heparinized tubes (Venoject®), and after 30 min at room temperature plasma was separated by centrifugation and was kept at -20 °C until analysed. Drug analysis was performed by HPLC [6].

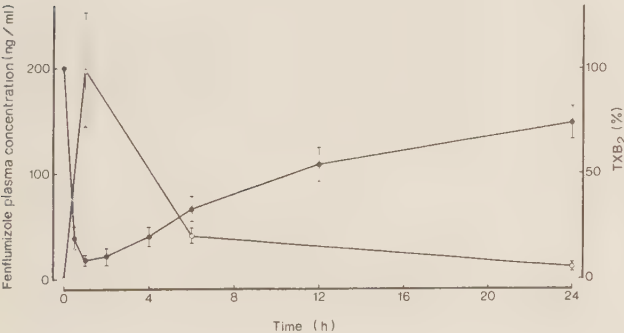


Fig. 4. Plasma concentration of fenflumizole after administration of 1 mg/kg (○—○) and formation of thromboxane B₂ in % of amount at zero h (●—●); mean ± SEM (n = 8)

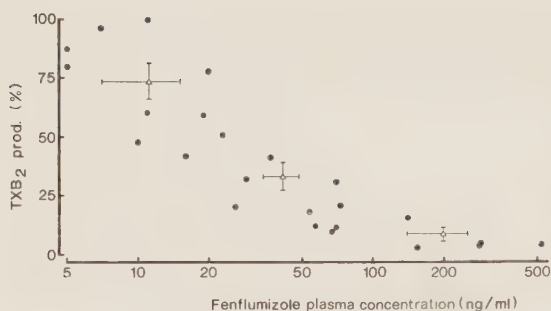


Fig. 5. Formation of thromboxane B_2 in serum after fenflumizole 1 mg/kg plotted against plasma concentration of the drug at 1, 6 and 24 h. 100% at 0 mg/kg (0 hours). Individual values and means $\pm$ SEM ($n=8$)

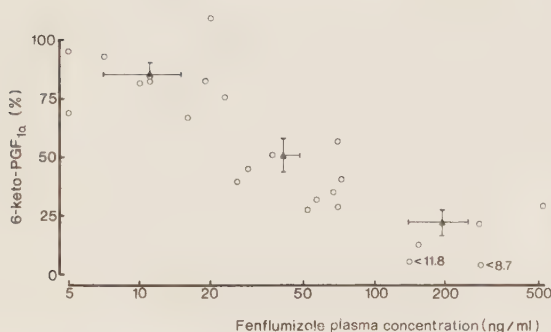


Fig. 6. Formation of 6-keto-prostaglandin $F_{1\alpha}$ in serum after fenflumizole 1 mg/kg against plasma concentration of the drug at 1, 6 and 24 h. 100% at 0 mg/kg (0 h). Individual values and means $\pm$ SEM, ($n=8$)

Formation of TXB_2 and 6-keto- $PGF_{1\alpha}$

The effect of fenflumizole on the formation of TXB_2 and 6-keto- $PGF_{1\alpha}$ in whole blood was studied after a single dose of fenflumizole of 1 mg/kg. The dose for this purpose was repeated under similar conditions several weeks after being first given. A modification of the method of Patrono et al. [7] for assessing TXB_2 -formation in clotting whole blood was applied. Blood was taken from an antecubital vein immediately before the dose and 0.5, 1, 2, 4, 6, 12, and 24 h after it, and was placed directly into siliconized tubes (Vacutainer®), which were incubated at 37°C for 1 h. Serum was separated and kept at -70°C until analysis. Samples of serum were then diluted 1:100 with 0.05 M phosphate buffer pH 7.4 and the TXB_2 -levels were assayed with a commercial radioimmunoassay (3H - TXB_2 , New England Nuclear, Boston, Ma, USA). Concomitant drug concentrations were measured in plasma samples withdrawn before the dose and after 1, 6 and 24 h. 6-keto- $PGF_{1\alpha}$ levels were measured in serum prepared as described above for TXB_2 . Serum samples were assayed directly for their contents of 6-keto- $PGF_{1\alpha}$ by means of radioimmunoassay (3H -6-keto- $PGF_{1\alpha}$, New England Nuclear).

Data Presentation

All results are given as mean $\pm$ sem, except when otherwise stated. For linear regression analysis the method of least squares was used. In the statistical analysis a p -value <0.05 was considered significant.

Results

Platelet Aggregation

As shown in Table 1, AA-induced platelet aggregation was affected after intake of fenflumizole at all three dose-levels. The anti-aggregatory activity of fenflumizole was already apparent 30 min after administration of each of the three doses. The maximum effect of the lowest dose was seen at 1–2 h and thereafter the effect declined. The anti-aggregatory effect following the doses of 1 and 2 mg/kg was at its maximum throughout the entire period of 8 h after administration during which aggregation was tested. Subject 1 appeared to have a different aggregatory response to AA from the other 7 subjects. As no initial threshold value could be established, he has been excluded from the analysis of data. No satisfactory

explanation of his different reaction pattern has been found.

For each sampling-time the calculated AA_{1min} (μM) was related to the drug concentration in plasma. The concentration-response relationship is illustrated in Fig. 3. A linear correlation between log drug concentration in plasma and AA_{1min} (μM) was found for each individual subject, even when results from all three doses were taken together. The individual r -values for the correlation ranged from 0.33 to 0.93 (mean $r = 0.74 \pm 0.074$, median 0.81; $p < 0.05$ except for the line of $r = 0.33$). Intraindividual changes in response to AA were generally small, but could explain the low r -value (0.33) in one subject, for whom the results at the highest dose deviated from those for the two lower doses. However, if the highest dose were omitted, the r -value for the correlation was 0.84, which was still not statistically significant.

Drug Concentration in Plasma

The maximum concentration of the drug in plasma was reached 1 h after administration and then rapidly decreased (Table 1). Detailed pharmacokinetic data will be presented elsewhere [8]. Plasma concentrations after the repeated 1 mg/kg dose are given in Fig. 4.

TXB₂-Formation

The mean TXB₂-level in serum after whole blood clotting before drug administration was 270 ± 40 ng/ml. If related to a standard platelet count in whole blood of $200 \times 10^9/l$, this would correspond to 246 ± 38 ng/ml. For each individual the effects of the 1 mg/kg dose of fenflumizole on TXB₂-formation were related to the level at 0 h, which was set at 100% (Fig. 4). Depression of the formation of TXB₂ was found in all the subjects. The lowest levels of TXB₂ were recorded 1–2 hours after the dose. At 24 h there had still not been a complete return to the starting-levels. The relationship between drug concentration and TXB₂-formation is presented in Figs. 4 and 5. At drug concentrations above 200 ng/ml, maximum depression of TXB₂-formation was attained. The EC_{50} was approximately 20 ng/ml.

6-keto-PGF_{1α}-Formation

The mean 6-keto-PGF_{1α}-level before treatment was 1.0 ± 0.05 ng/ml, and it decreased after administration of the drug; the relation to the concomitant drug concentration is shown in Fig. 6. As for TXB₂, the levels are expressed as % of those measured immedi-

ately before the administration of fenflumizole. The EC_{50} was approximately 40 ng/ml.

Adverse Reactions

There were no major changes in blood pressure or heart rate following the administration of fenflumizole. No adverse reaction attributable to the drug was observed clinically or in laboratory tests.

Discussion

All three doses of fenflumizole were rapidly absorbed from the gastrointestinal tract. Platelet aggregation induced by AA was affected when the drug was present even at a very low concentration close to the lower limit of measurement (0.5 ng/ml). The shape of the aggregation tracings changed considerably, which made interpretation of the effects difficult.

Originally, the intention was to follow the change in the concentration of AA required to induce irreversible aggregation at different times after dosage. This threshold concentration was always increased after the drug had been taken. However, in several samples the threshold concentration could not be identified. This might have been due to the bell-shaped dose-response curve for AA on the increase in light transmission following platelet aggregation, which may reflect the formation of lipoxigenase products, or a direct physical interaction between AA and the platelet membrane [9]. It might also be attributed to the actions of fenflumizole on metabolism of endogenous AA *in vivo*.

When AA was added to drug-free PRP to give a final concentration of 1500–2000 μM , aggregation was often reversible, and when the drug was present in plasma the reversibility tended to occur at lower concentrations. Below these concentrations it was still possible to induce irreversible aggregation in some but not all PRP samples, when the threshold level was increased. Therefore, a progressive decrease in the maximum light transmission through PRP could not be used as a parameter of antiaggregatory effect. Instead, the lag phase between addition of AA to PRP and the start of the aggregatory response was recorded and related to the plasma concentrations of fenflumizole. The lag phase was prolonged or non-identifiable in the presence of the drug, but this could be overcome by increasing amounts of AA. The degree of prolongation of the lag phase was found to correlate well with drug concentration in plasma.

It has been maintained that certain fatty acids and AA metabolites of the lipoxygenase pathway can have an antiaggregatory effect on platelets [10, 11]. This has been found, for example, for 12-HPETE [12]. In the presence of excess substrate (AA), or if the cyclo-oxygenase pathway is blocked, HPETE may be produced in amounts that could decrease the aggregatory response to AA. On the other hand, it has been proposed that the lipoxygenase-derived metabolites contribute to the irreversibility of AA-induced platelet aggregation, in which case inhibition of the lipoxygenase activity would give reversible aggregation [11, 13]. It cannot be excluded that the reversible aggregation observed in the present study is attributable to changes in the formation of lipoxygenase products by fenflumizole and/or by large amounts of AA (self-destruction of the enzyme).

TXB₂-formation in clotting whole blood was greatly inhibited by fenflumizole. The method used evaluates the potential for TXB₂-formation by platelets and possibly other formed blood elements and gives no information on circulating TXB₂-levels. However, it is useful for studying changes in endogenous AA metabolism in this tissue. The TXB₂-levels found at zero time were in accordance with those reported in other studies using similar methods [7, 14, 15, 16]. After treatment the profile of the TXB₂-curves mirrored the plasma concentration curves, and it was found that TXB₂-formation was maximally inhibited when fenflumizole concentrations exceeded 200 ng/ml.

The 6-keto-PGF_{1α}-levels in serum were low when compared to those of TXB₂, but the circulating level of this prostanoid is thought to be even lower [17, 18]. The present results may reflect the fact that other metabolites of AA can cross-react to some degree with the antibodies of the RIA, but it may also indicate the formation of 6-keto-PGF_{1α} in blood, possibly by leukocytes [19, 20]. In the presence of dazoxiben, an inhibitor of TXA₂-synthetase, the levels of 6-keto-PGF_{1α} (and prostaglandin E₂) in whole blood have been found to increase, while TXB₂-levels drop [19, 20]. This was not found to be the case for fenflumizole, supporting earlier findings that this substance does not inhibit TXA₂-synthetase [2]. If cyclo-oxygenase is inhibited, the 6-keto-PGF_{1α}-levels should fall following dosage, which is the case for fenflumizole (present results). The same effect has been observed for aspirin and indomethacin (Vinge, unpublished results).

In the present study, effects on the aggregation of platelets by AA in vitro and the formation of TXB₂ in clotting whole blood were found to be correlated with the plasma concentration of the drug. The

greatest changes in effect were seen at plasma levels up to about 100 ng/ml. The two methods used may reflect the same pharmacological effect of fenflumizole. When other substances have been tested, effects on platelet aggregation can be shown to be dissociated from effects on TXB₂-formation [21], but this was not found for fenflumizole in the present study.

It is well known that several different pathways are important in platelet aggregation, and a number of biochemical mechanisms are involved. When platelet aggregation is triggered by one substance, a cascade of biochemical events follows. AA-induced platelet aggregation and TXB₂-measurement in serum may be used to assess the effect of drugs that interfere with formation of prostanoids and/or leukotrienes. However, conclusions regarding the anti-aggregatory effects of fenflumizole in vivo cannot be based solely on these results.

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Arachidonic Acid – Induced Platelet Aggregation and Prostanoid Formation in Whole Blood in Relation to Plasma Concentration of Indomethacin

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Summary. A single oral dose of indomethacin 1 mg/kg was given to 6 male and 6 female volunteers. The formation of thromboxane B₂ (TXB₂) and 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) in clotting whole blood was measured by radioimmunoassay, and platelet aggregation induced by arachidonic acid (AA) was measured with a plasma aggregometer. The results were related to the concomitant plasma concentration of indomethacin. The maximum plasma concentration ranged between 3.24 and 8.11 µg/ml and the elimination half-life between 4 and 11 h. Formation of the prostanoids was reversibly inhibited, with maximum suppression when the drug concentration in plasma exceeded 0.5–1.0 µg/ml; the IC₅₀ was approximately 0.1 µg/ml. Platelet aggregation was also reversibly inhibited. The correlation between the formation of prostanoids and the different phases of the aggregatory response to exogenous AA is discussed.

Key words: indomethacin, platelet aggregation, prostanoids; plasma concentration, arachidonic acid, thromboxane B₂, 6-keto-prostaglandin F_{1α}, kinetics

strate irreversible inhibition of the enzyme cyclooxygenase [3]. Others, however, have found evidence for competitive inhibition of prostanoid formation by indomethacin in vivo, by studying AA-induced platelet aggregation and thrombin-stimulated prostaglandin E₂ release [4]. In both studies aggregation was measured as the threshold level of exogenous AA, i.e. the lowest concentration required to induce irreversible aggregation.

In addition to threshold levels, the platelet aggregatory response to exogenous AA can be measured as the degree of light transmission through a platelet suspension [5, 6], as the maximum rate of change in light transmission [7], the time to reach 50% of the maximal alteration in light transmission [8] or as the rate of change of shape [9]. In a previous study of a new inhibitor of prostaglandin synthesis, fenflumizole, a close correlation was found between the length of the lag-phase in the aggregatory response to AA and the drug concentration in plasma [10]. In many of the samples it was not possible to determine any threshold for irreversible aggregation, which might have been due to the bell-shaped dose-response to AA in light transmission alteration [6]. Formation of the prostanoids thromboxane B₂ (TXB₂, metabolite of thromboxane A₂) and 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}, metabolite of prostacyclin) in whole blood [11] was inhibited in a concentration-dependent manner and the EC₅₀ was established. However, to the best of our knowledge no comparable data on the lag phase relations are available for any reference drug, and data on the concentration-response relationship for prostanoid formation in whole blood are incomplete.

Therefore, the present study was designed to provide more information on the aggregatory response to AA in relation to different plasma concentrations of indomethacin. In addition, the formation of TXB₂

Since 1971 [1, 2] indomethacin has been used as a model drug and reference substance for studying the inhibition of formation of prostanoids from arachidonic acid (AA) in different tissues. Prostanoids appear to play a major role in normal platelet function, and many substances known to trigger platelet aggregation also release AA from membrane phospholipids making it available for metabolism into endoperoxides and thromboxanes.

The effect of indomethacin added in vitro to platelet suspensions has been reported to demon-

and 6-keto-PGF_{1 α} in whole blood was measured by radioimmunoassay and was related to the concomitant drug concentration. In order to screen for a possible influence of sex on the platelet reactions, the drug was given both to men and women.

Materials and Methods

Subjects

Twelve subjects, 6 men and 6 women, volunteered for the study. Their mean ages were 30 years (range 25–35 years) and 37 years (range 30–46 years), respectively. They were all considered healthy in terms of clinical examination, including blood chemistry and haematology. None had taken any medication during the week preceding the trial, and all abstained from alcoholic beverages for 24 h before and during the trial day. All subjects gave their written informed consent, and the study was approved by the Ethics Committee of the University of Lund, Sweden.

Procedure

Indomethacin 1 mg/kg (Dumex Ltd, Copenhagen, Denmark) was given orally with 100 ml water to each subject, in the morning after an overnight fast. The drug was administered as an aqueous suspension at 5 mg/ml, prepared with Tween 80 1 mg/ml, traga-canth 3.75 g/ml, sorbic acid 0.75 mg/ml and ethanol 3.75 mg/ml. After 3 h a light meal was served. After 4 h a regular diet was allowed. Blood was collected from an antecubital vein prior to, and 1/2, 1, 2, 4, 6, 12 and 24 h after drug administration for analysis of drug concentration in plasma and the formation of thromboxane B₂ and 6-keto-prostaglandin F_{1 α} in whole blood. Blood for platelet aggregation was collected at 0, 1, 6 and at 12 or 24 h.

Drug Concentration in Plasma

Heparinized venous blood was collected in vacuum tubes (Venoject), and the plasma was separated and kept at –20°C until analysed. The drug was determined by gas chromatography of the ethyl derivate of indomethacin, using an electron capture detector, as described by Jensen [12].

Formation of Thromboxane B₂ and 6-Keto-Prostaglandin F_{1 α}

Venous blood was collected in siliconized tubes (Vacutainer), and was allowed to clot at 37°C for 1 h. Serum was then separated and was kept in polypro-

pene caps at –20°C until analysed for its content of prostanoids. Thromboxane B₂ and 6-keto-prostaglandin F_{1 α} were determined by radioimmunoassay, using commercially available kits (³H-Thromboxane B₂-RIA and ³H-6-keto-Prostaglandin F_{1 α} -RIA, New England Nuclear, Boston, Mass., USA). Serum was diluted 1:100 with phosphate-buffer 0.05 M, pH 7.4, for thromboxane B₂-analysis, and undiluted serum was used for the 6-keto-prostaglandin F_{1 α} assay. Standards were made up in buffer. 0.1 ml standards or samples were mixed with tracer, antibody and buffer to a final volume of 1 ml, and was incubated overnight at 4°C. In standard vials 100 μ l buffer was replaced by an equal volume of human 0-serum, diluted 1:100 or undiluted when appropriate, to compensate for the serum in the unknown samples. Thromboxane B₂-controls were made up in 0-plasma, stored at –20°C and processed in both assays in the same way as the unknown samples to check for recovery and cross-reactivity.

Platelet Aggregation

Venous blood was immediately mixed with sodium citrate 0.129 M in the proportion 9:1, and centrifuged at 190 $\times$ g for 15 min to yield platelet rich plasma (PRP). Platelet poor plasma (PPP) was obtained by further centrifugation of the sample at 1000 $\times$ g. Aggregation was studied in a HU-aggregometer (37°C, stirred at 1100 rpm) connected to a Vitatron recorder. Light transmission through PRP was set to 10 and through PPP to 90. Aggregation was induced by adding different amounts of arachidonic acid (AA; final concentrations 0.3, 0.5, 1.0, 1.5 or 2.0 mM) to PRP. Arachidonic acid (Nu-Chek-Prep, Elysian, MN, USA) was dissolved in ethanol and sodium bicarbonate was added (AA 50 mg, ethanol 1.4 ml, Na₂CO₃ 6.1 ml).

Adverse Effects

Side-effects of the drug were recorded in a separate protocol, both as spontaneous reports and after active questioning of the subjects.

Results

Plasma Concentration of Indomethacin

The maximum plasma concentration of indomethacin was seen 0.5–1 h after drug intake and ranged from 3.24 to 8.11 μ g/ml (median 4.64 μ g/ml). The pharmacokinetic profile could be described by a

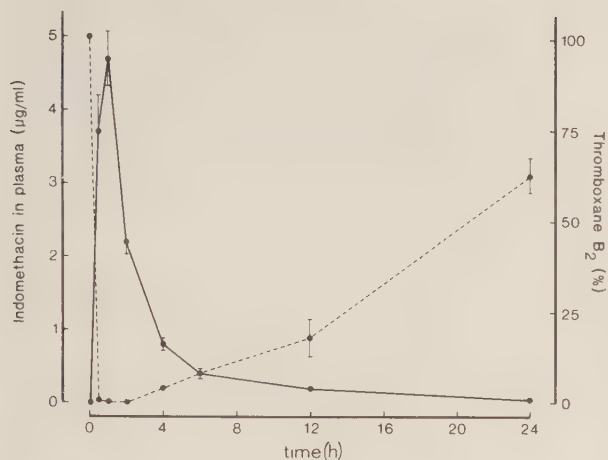


Fig. 1. Plasma concentration of indomethacin (—●—) and formation of thromboxane B_2 in % of control value at 0-time (---●---) during 24 h after ingestion of indomethacin 1 mg/kg. Mean $\pm$ SEM, $n = 12$

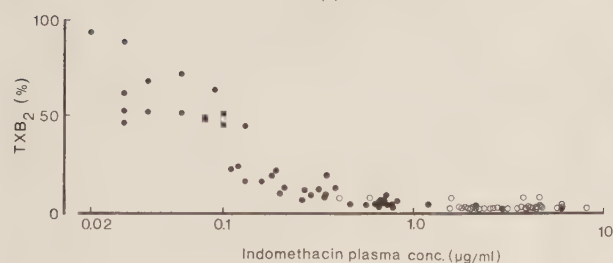


Fig. 2. Formation of TXB_2 -like activity in serum ($37^\circ C$) in % of the predosing value (= at 0-time) related to concomitant plasma-concentration of indomethacin after a single dose. Individual results for 12 subjects. In cases where the TXB_2 -level fell below the limit of detection an empty circle marks the latter

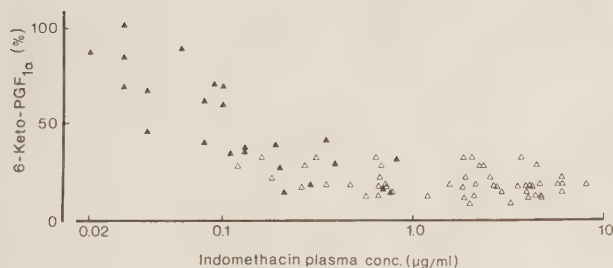


Fig. 3. Formation of 6-keto- $PGF_{1\alpha}$ -like activity in serum ($37^\circ C$) in % of the predosing value (= at 0-time) related to concomitant plasma concentration of indomethacin after a single dose. Individual results for 11 subjects. In cases where the 6-keto- $PGF_{1\alpha}$ -level fell below the limit of detection an empty triangle marks the latter

two-compartment model (Fig. 1). The plasma clearance was 0.043 to 0.094 l/kg $\cdot$ h, and the plasma half-life of the drug in the elimination phase ranged from 4 to 11 h (median 6 h).

Prostanoid Formation

At 0 h the mean ($\pm$ SEM) level of TXB_2 -like activity in serum was 260 ± 50 ng/ml for women and 249 ± 44 ng/ml for men, with a total range of 66–500 ng/ml (limit of detection 5 ng/ml). The corresponding lev-

els for 6-keto- $PGF_{1\alpha}$ -like activity were 0.70 ± 0.06 ng/ml and 0.50 ± 0.17 ng/ml, with an overall range from below the detection limit (0.10 ng/ml) to 1.25 ng/ml. Formation of both prostanoids was depressed when indomethacin was present in plasma. The lowest values were seen at 0.5–2 h after drug intake, but as indomethacin was progressively cleared from plasma increasingly higher levels of both prostanoids were found (Figs. 1, 2, 3). 6-Keto- $PGF_{1\alpha}$ levels were usually below the limit of detection when the indomethacin concentrations was higher than 0.5

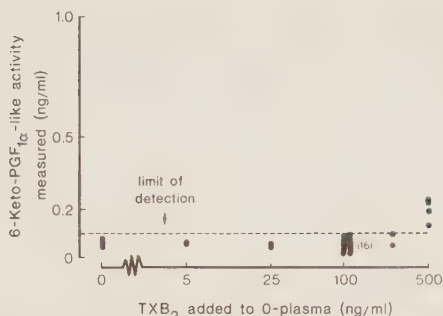


Fig. 4. Cross-reactivity in the 6-keto-PGF_{1α}-assay. Undiluted samples of TXB₂-controls made up in 0-plasma were processed in the same manner as unknown samples

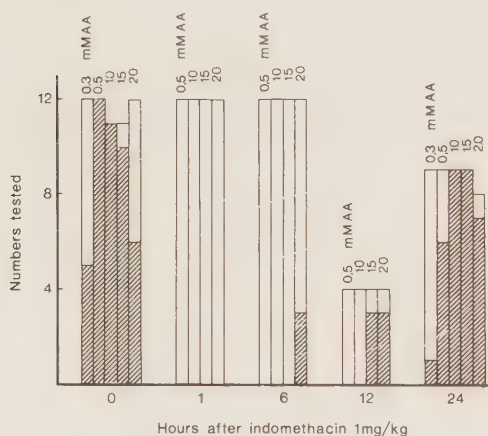


Fig. 5. Appearance of irreversible platelet aggregation as a response to exogenous AA at different concentrations, before and following a single dose of indomethacin. Hatched bars = irreversible aggregation within 5 min. Unfilled bars = reversible aggregation or no reaction

μg/ml, and TXB₂ was undetectable at indomethacin concentrations > 1.0 μg/ml. Thus, high indomethacin concentrations did not give measurable immunoreactivity. Controls for TXB₂ 500 ng/ml in 0-plasma could be measured as approx 0.2 ng/ml 6-keto-PGF_{1α}-like activity (< 0.1%) but not at concentrations of 250 ng/ml or lower (Fig. 4), which confirms the cross reactivity data presented by the manufacturer. No qualitative difference in response could be detected between men and women.

Production of thromboxane B₂ and 6-keto-prostaglandin F_{1α}, expressed as % of the levels before drug administration (0 h) for each subject, plotted against the concomitant concentration of indometh-

acin in plasma are presented in Figs. 2 and 3. The one case with no detectable 6-keto-PGF_{1α} has been excluded from Fig. 3. In some cases the limit of detection of 6-keto-PGF_{1α} was 25–30% of the level at 0 h. The concentration of indomethacin at which the formation of either prostanoïd was inhibited by 50% (IC₅₀) was approximately 0.1 μg/ml (2.6×10^{-7} M).

Platelet Aggregation

A pronounced impairment of platelet aggregation induced by AA was always seen after intake of indomethacin, but it was reversible and declined towards the end of the 24-h observation period.

Aggregation, measured as a complete and irreversible aggregatory response to different concentrations of AA, is presented in Fig. 5. The threshold value, i.e. the lowest concentration of AA which would elicit irreversible aggregation, was always 0.5 mM or lower when no indomethacin was present. However, when the AA-concentration in PRP was 1.5 or 2.0 mM, aggregation was sometimes reversible even in the absence of indomethacin. The presence of indomethacin inhibited the aggregatory response, albeit not completely, as the inhibition could partly be overcome by a higher concentration of AA in PRP. Aggregation was usually abolished when platelets were challenged with 0.5 mM AA, but when higher concentrations of AA were used, various degrees of the aggregatory response were seen.

In normal plasma the length of the lag phase between addition of AA to PRP and the start of the increase in light transmission is dependent on the amount of AA added. In presence of indomethacin the lag phase either did not occur within 5 min, or was only slightly prolonged (in some cases it was unchanged), depending on the concentration of AA used. Median values for the lag phase at each AA-concentration at the different sampling times are presented in Fig. 6. The AA-concentration required to produce a standard lag phase of 1 min (AA_{1 min}) was estimated and was correlated with the plasma concentration of indomethacin (Fig. 7). Administration of vehicle alone to 2 subjects did not change the lag phase over 6 h.

Adverse Effects

Eight of the 12 subjects reported dizziness, which occurred 0.5–1 h after dose administration and usually lasted for less than 2 h. Two of these subjects also reported nausea, and one had headache and gastrointestinal discomfort in addition.

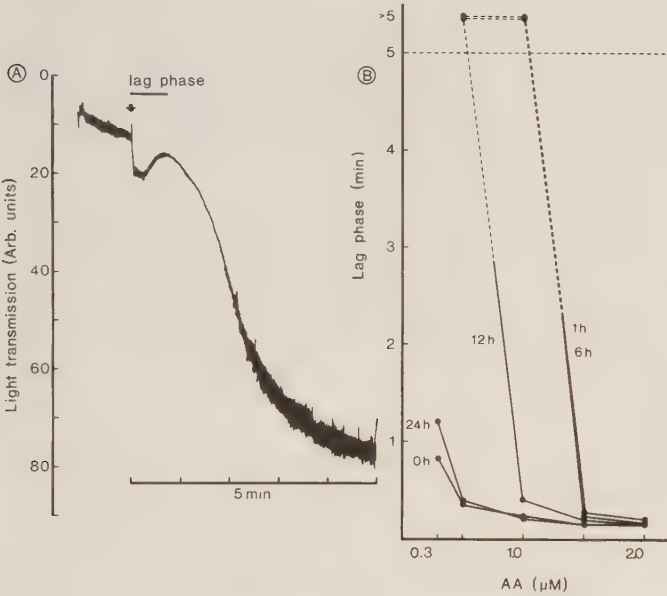


Fig. 6. a Tracing of irreversible aggregation induced by 0.5 mM AA in absence of drugs. b Length of the lag phase between addition of AA to the platelet suspension and the start of the increase in light transmission as a function of the concentration of AA. Median values for samples tested at 0 h ($n = 12$), 1 h ($n = 12$), 6 h ($n = 12$), 12 h ($n = 5$) and 24 h ($n = 9$)

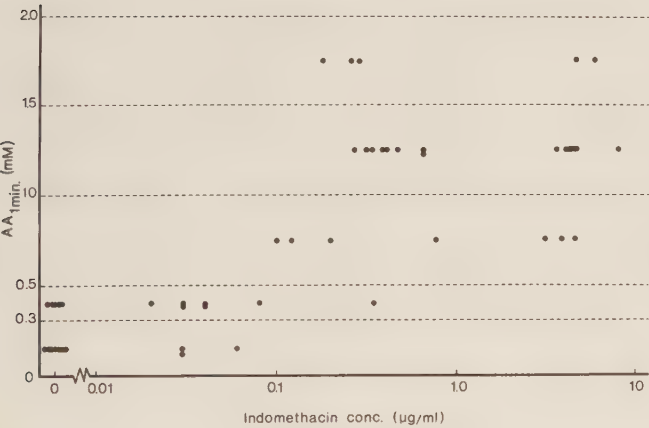


Fig. 7. Estimated concentration of AA (in intervals) needed to give a standard lag phase of 1 min in the platelet aggregatory response to AA related to the concomitant plasma concentration of indomethacin. Individual results for 12 subjects

Discussion

The present results show a concentration-dependent, reversible inhibitory effect on prostanoid formation and platelet aggregation of indomethacin. Complete inhibition of prostanoid formation was obtained at drug concentrations above 1 μg/ml. Inhibition of aggregation could be overcome by adding an increased amount of AA. These observations accord with those of Rane et al. [4].

The drug concentrations achieved, and the calculated values for clearance and elimination half-life, agree with those reported previously [13]. It was noted that side-effects, mainly dizziness, occurred when the plasma concentration was highest, and then usually for a limited period. Two subjects were more affected than the others, one of whom in fact reached the highest peak value in the study.

The data from measurement of prostanoids suggest that inhibition of formation can be described by

a sigmoidal function of the logarithm of drug concentration, with maximum inhibition being reached at a plasma level of the drug > 0.5 – 1.0 $\mu\text{g/ml}$. This corresponds well with the effects on thrombin-stimulated PGE_2 -release reported by Rane et al. [4]. After in vitro incubation of human platelets with indomethacin, the IC_{50} for inhibition of prostanoid synthesis, measured as malondialdehyde(MDA)-production, was reported to be 2×10^{-6} M (0.72 $\mu\text{g/ml}$); [14]. When measured as TXB_2 -formation from platelets aggregated with collagen, the IC_{50} for indomethacin was 1×10^{-7} M (0.04 $\mu\text{g/ml}$) [15]. In the present study, the IC_{50} of indomethacin was approximately 0.1 $\mu\text{g/ml}$ (2.7×10^{-7} M). The value was the same for inhibition of the formation of TXB_2 and 6-keto- $\text{PGF}_{1\alpha}$, which would be expected from a cyclo-oxygenase inhibitor, but not for a drug with a selective effect on thromboxane synthetase, like dazoxiben. Indeed, it has been reported that the latter drug augments the formation of 6-keto- $\text{PGF}_{1\alpha}$ in clotting whole blood while TXB_2 -formation is depressed [16, 17, 18, 19]. This finding indicates that 6-keto- $\text{PGF}_{1\alpha}$ in serum is not simply a phenomenon of cross-reactivity between TXB_2 and antibodies against 6-keto- $\text{PGF}_{1\alpha}$ in the radioimmunoassay. In our assay the undiluted controls of TXB_2 in plasma ≤ 250 ng/ml gave no detectable immunoreactive 6-keto- $\text{PGF}_{1\alpha}$, and only 0.2 ng/ml in the TXB_2 -control of 500 ng/ml. However, cross-reactivity towards other prostanoids is probable, e.g. PGE_2 , $\text{PGF}_{2\alpha}$ and PGD_2 [16, 18, 19]. The degree of cross reactivity depends on the specificity of the antibody used, but immunoassays will tend to overestimate the absolute value of 6-keto- $\text{PGF}_{1\alpha}$ in serum, as stressed by Pedersen et al. [17]. Nevertheless, the relative changes in immunoreactivity in presence of a drug may be of interest.

Platelet aggregation induced by adding exogenous AA to PRP was changed after administration of indomethacin. At 0 h (before treatment), the bell-shaped response to AA appeared when development of irreversible aggregation was considered, but not when the lag-phase was measured. In the presence of indomethacin the aggregability of platelets diminished but gradually returned to normal as the drug was cleared from plasma. Between 1 and 6 h, little change occurred in the response pattern, which probably reflects close to maximum inhibition of the system during the period when the mean plasma concentration exceeded 0.5 – 1.0 $\mu\text{g/ml}$. The highest AA-concentration used could overcome the inhibition, at least in part, which is consistent with competition between AA and indomethacin for the same receptor, in this case the enzyme.

In presence of indomethacin, the lag phase was

either not identifiable within 5 min after addition of AA to the platelet suspension, or it was only slightly prolonged. This differs from the gradual prolongation of the lag phase seen in the presence of fenflumizole, a previously studied inhibitor of prostaglandin synthetase [10]. The discrepancy may be explained by a steeper concentration-response curve in presence of indomethacin. Narrower intervals of AA-concentrations would then have revealed successive prolongation of the lag phase. Another possibility is an all-or-none response when indomethacin is present, in contrast to fenflumizole, which would indicate a difference in the mechanism of action of the two drugs. If so, the lag phase may not represent prostanoid formation but rather some other step in AA metabolism, possibly formation of lipoxigenase products.

If a standard lag-phase time is estimated from the AA-lag phase time curves, e.g. a lag phase of 1 minute, and this value is related to the concomitant concentration of indomethacin in plasma, the relationship between the degree of inhibition and the indomethacin concentration can be described. A plot of the individual data suggests increased inhibition up to a concentration of indomethacin of 0.5 – 1.0 $\mu\text{g/ml}$, which seems to accord well with the inhibition of prostanoid formation in clotting blood (Fig. 6). There were differences between individual subjects with respect to maximum response and the slope of the curve, as noted previously [10]. A similar observation in 5 healthy men was made by Rane et al. [4]. Determination of the lag time to 50% change in light transmission, which is dependent both on the time for shape change and the rate of change in light transmission, has also been found to be correlated with platelet TXA_2 production [8]. Patscheke and Stegmeier [9] studied the shape change phenomenon, and found that a thromboxane receptor antagonist (BM 13.177) inhibited the shape change and aggregation induced by hydrogen peroxide, AA and endoperoxide analogues. Their results indicate that the lag phase (or rate of shape change) in AA-induced aggregation is correlated with the agonistic effects of endoperoxides and thromboxanes, rather than with their formation from AA.

In the present study threshold levels of AA for irreversible aggregation could have been used as a measure of platelet activity. However, as challenge of platelets with AA-concentrations of 1.5 or 2.0 mM in some cases gives spontaneously reversible aggregation, even in the absence of any drug, the extent of the inhibitory effect exerted by indomethacin is not clear.

The present results suggest that measurement of prostanoid formation in whole blood is a simple

method to establish concentration-response curves for cyclo-oxygenase inhibitors given to humans, both men and women. When turbidimetric studies of AA-induced platelet aggregation are performed, the lag phase in aggregation tracings may be a better index of prostanoid inhibition than threshold level or change in light transmission.

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COMPARED EFFECTS OF THE TWO ANTIINFLAMMATORY DRUGS INDOPROFEN AND
LONAZOLAC ON THROMBOXANE GENERATION AND ON PLATELET AGGREGATION,
RELATED TO PLASMA CONCENTRATIONS AFTER SINGLE ORAL DOSES.

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SUMMARY

Single doses of indoprofen 100 mg and lonazolac-Ca 200 mg were given orally to eight healthy volunteers. Blood samples were taken prior to and at various times until 24 hours after the dose. Drug concentrations in plasma were analyzed by HPLC and thromboxane B₂ (TXB₂) in serum by radioimmunoassay. Platelet aggregation in plasma was induced by various concentrations of arachidonic acid and evaluated with respect to different phases in the aggregatory response. The drugs were rapidly absorbed and for both compounds the decline in plasma concentrations described two phases. Although concentrations of indoprofen were up to ten times higher than those of lonazolac, the two drugs were almost equally potent inhibitors of generation of TXB₂, with IC₅₀ for indoprofen of 1.4-1.5 x 10⁻⁷M and for lonazolac 2.1-3.0 x 10⁻⁷M. Their effects on the lag phase in platelet aggregation paralleled the inhibition of TXB₂ formation. However, the rate and extent of aggregation were more inhibited by indoprofen than by lonazolac, and the discrepancy was greater than would be expected from concomitant values for TXB₂ suppression. The rate of aggregation appeared to be the most sensitive variable, since the extent of aggregation followed a all-or-none pattern. The present results show that the concentrations reached after clinically used doses of anti-inflammatory drugs may vary widely in relation to the concentrations needed for maximum inhibition of platelet cyclooxygenase activity. In addition, the complex aggregatory response to exogenous arachidonic acid is discussed. The results may support the idea that nonsteroid antiinflammatory drugs can differ in their mechanisms of action.

Key words: Antiinflammatory drugs, indoprofen, lonazolac, thromboxane B₂, platelet aggregation, pharmacodynamics

INTRODUCTION

With a few exceptions, non-steroidal antiinflammatory drugs are known to inhibit platelet aggregation, due to their inhibitory effect on the conversion of arachidonic acid into metabolites of the cyclooxygenase pathway (Smith & Willis 1971, Cronberg et al. 1984). However, the extent of inhibition of platelet aggregation is dependent on the proaggregatory substance used, and its concentration (De Caterina et al. 1985). Certain proaggregatory agents are able to induce aggregation via pathways independent of formation of prostanoids (Vargaftig et al. 1981). Exogenous AA can induce platelet aggregation (Vargaftig & Zirinis 1973) which is believed to occur via formation of endoperoxides and thromboxane A_2 (Hamberg et al. 1974, 1975), and is usually accompanied by, but not dependent on, release of proaggregatory substances from platelet granules (Kinlough-Rathbone et al. 1976, Burch & Lamb 1983). The magnitude of the aggregatory response to AA shows a bell-shaped pattern (Linder et al. 1979, Fratantoni & Poindexter 1981, Aharony et al. 1982, Vinge 1985), and it has been suggested that AA-metabolites of the lipooxygenase pathway may be involved and interact with endoperoxides and thromboxanes resulting in a decreased aggregatory response (Aharony et al. 1982).

We have previously studied the effects on platelet aggregation and thromboxane formation after oral doses of indomethacin and fenflumizole to volunteers (Vinge et al. 1984, Vinge 1985). These compounds possess different chemical structures, but a common ability to inhibit prostanoid formation (Corell et al. 1983). Both were potent concentration-dependent inhibitors of TXB_2 -formation in blood and of platelet aggregation induced by AA, but the results suggested that they did not influence aggregation in identical ways. Although their effects on the lag phase of aggregation appeared to be similar, the results on the rate and extent of aggregation appeared to differ. This raised the question whether aggregation tracings may reflect other properties of anti-inflammatory drugs than their potency to inhibit the cyclooxygenase enzyme.

The aim of the present study was to compare another two non-steroidal antiinflammatory drugs with special reference to their abilities to reduce the thromboxane forming capacity in blood and to alter platelet aggregatory response induced by exogenous arachidonic acid. A secondary aim was therefore to characterize the aggregatory response to various concentrations of AA in absence and presence of antiinflammatory drugs in the body, with special reference to the lag phase. The compounds chosen were indoprofen and lonazolac. The former is a representative of the propionic acid derivatives (Ceserani et al. 1979), which include antiinflammatory compounds like ibuprofen, naproxen and ketoprofen which have been in clinical use for several years. Lonazolac is a pyrazole acetic acid derivative (Rainer et al. 1981), which has been found to posses high antiinflammatory potency, but less toxicity than reference drugs (Riedel 1981). Both indoprofen and lonazolac were subjects for clinical investigations with regard to antiinflammatory and analgesic properties, but data on their anti-platelet effects in man in relation to concentration in plasma were lacking.

METHODS

Drugs

Indoprofen (Farmitalia, Carlo-Erba, Milano, Italy) was used in capsules of 100 mg, and lonazolac as its calcium-salt in tablets of 200 mg (Lonazolac-Ca, Irritren^R, BykGulden, Konstanz, Germany).

Procedure

Both drugs were given as single oral doses to 8 healthy subjects, 3 men and 5 women, with a mean age of 36.5 years (range 26-46 y) and a mean body weight of 66.9 kg (range 53-78 kg). All had previously taken part in a similar study with indomethacin. The subjects were not allowed to take any drugs with known effects on prostaglandin generation or on platelet function for 8 days before each trial day. Alcoholic beverages and smoking was prohibited on trial days.

Each drug was given in the morning with 100 ml of water to the subject, who had fasted overnight. A standardized breakfast was served after 2 hours. There were at least two weeks wash-out between the trial days for each subject.

For measurements of drug concentrations in plasma and thromboxane formation in serum, blood was collected immediately before the drug administration (=0 hours) and at 1, 2, 4, 6, 8, 12 and 24 hours after. For platelet aggregation blood was sampled at 0, 2, 6 and 24 hours.

The protocol was approved by the Ethics Committee of the University of Lund, Sweden.

Drug concentrations in plasma

Plasma from heparinized blood collected in vacuum tubes (Venoject^R) was stored at -20° C until analysis.

Indoprofen was determined by high performance liquid chromatography (HPLC) (Wåhlin-Boll et al. 1981). By the use of two 25 cm LiChroCart RP-18 columns coupled in series and eluted with methanol-

phosphate buffer, 0.01 M, pH 3.5 (17:10) it was possible to detect indoprofen down to plasma levels of 10 ng/ml, and to quantitate it at plasma levels exceeding 40 ng/ml (1.4×10^{-7} M).

Lonazolac and its main metabolite, a p-hydroxylated metabolite, were determined by HPLC with some modifications of the method described by Huber et al. (1982). The analytical column was a Hypersil RP-8 5 μ m (Shandon). Concentrations above 250 ng/ml were evaluated with UV-detection, whereas fluorescence was used for lower levels. The detection limits were 7 ng/ml (2.2×10^{-8} M) for lonazolac and 3 ng/ml (9.1×10^{-9} M) for the metabolite when fluorescence detection was used (Huber & Zech, personal communication).

TXB₂-formation

Five ml of blood was collected in silicone-coated glass tubes and were allowed to stand at 37°C for one hour (Patrono et al. 1980), after which serum was collected and kept at -20°C until analysis of immunoreactive TXB₂ by radioimmunoassay (RIA). All serum samples were diluted with buffer 1:100. RIA-kits were purchased from New England Nuclear (Boston, Mass., USA; for details on the analytical procedure, see Vinge 1985).

Platelet aggregation by arachidonic acid

For platelet aggregation 18 ml of blood was immediately mixed with 2 ml of sodium-citrate 0.129 M. After centrifugation at 190 x g for 15 min platelet rich plasma (PRP) was separated, and after further centrifugation at 1000 x g platelet poor plasma (PPP) was obtained. Platelets in PRP were counted by use of a Burkner chamber, but the platelet number was not adjusted, as previous experiments had shown very little influence of platelet count on the lag phase of aggregation (unpublished). Aggregation was studied at 37°C in a HU-aggregometer, stirring speed 1100 rpm, and tracings were recorded by a Vitatron recorder. Light transmission (LT) through PRP and PPP was set at 10 and 90 on the chart scale, respectively. The chart was run at 20 mm/min. Arachidonic acid

from Nu-Chek-Prep (Elysian, Mn, USA) was dissolved in ethanol and Na_2CO_3 (Vinge et al. 1984), protected from light and was kept overnight under nitrogen at $+4^\circ\text{C}$.

Platelet aggregation was tested with various concentrations of AA, usually including 0.5, 1.0, 1.5 and 2.0 mM. The aggregation tracings were analyzed with respect to different events in the aggregation curves: a) the lag-time to the end of shape change and the start of increase in LT (minutes), b) the rate of aggregation expressed as maximum slope in the first phase of the light increase (scale units per minute), and c) the change in LT (LT, scale units) from before addition of AA to after 5 min, and d) the appearance of irreversible aggregation (Fig 1).

Adverse effects

All side-effects reported by the subjects, either spontaneously or after questioning, were recorded in separate protocols for each drug. Laboratory tests of hematologic, renal and hepatic functions were done before the study and 2 days after each drug administration.

Data processing:

In cases where concentrations of drug in plasma or of TXB_2 in serum were below the limit of determination, the values for this limit has been used for calculation of means.

For statistical calculations the Wilcoxon signed ranks test (two-tailed) was performed. Linear regression analysis was performed by use of the method of least squares. Values for $p < 0.05$ were regarded as statistically significant.

RESULTS

Drug concentrations in plasma

Median concentrations in plasma of each drug are shown in Fig 2. Both indoprofen and lonazolac were rapidly absorbed from the gastrointestinal tract. The maximum concentration of indoprofen was found at 1 h (5 subjects) or at 2 h (3 subjects), whereas the highest concentration of lonazolac was always found at 1 h. This was also true for its hydroxylated metabolite except for one subject. The maximum concentrations of indoprofen ranged from 6.2 to 12.0 $\mu\text{g/ml}$ which corresponds to $22.0 - 42.7 \times 10^{-6}$ M. Lonazolac maximum concentrations ranged from 1.1 to 5.2 $\mu\text{g/ml}$ ($3.5 - 16.7 \times 10^{-6}$ M), and the maximum concentrations of the metabolite were 0.63 to 2.4 $\mu\text{g/ml}$ ($1.9 - 7.2 \times 10^{-6}$ M). For one of the subjects (subject nr 2) the plasma-concentrations of lonazolac in the elimination phase (but not of its metabolite) were 5-10 times higher than those seen in the other 7 subjects.

For both indoprofen and lonazolac the concentration decline in plasma described two phases, with the terminal phase usually distinguished after 6-8 hours. The elimination half-life was estimated graphically to 3.2 - 6.2 (median 4.2 h) for indoprofen and to 6.0 - 19.6 h (median 8.4 h) for lonazolac.

After 24 hours the median indoprofen concentration was 1.96×10^{-7} M, the median level of lonazolac 2.7×10^{-8} M and of its metabolite 8.5×10^{-8} M.

TXB₂-formation

In drug-free serum the TXB₂-immunoreactivity was 212 ± 18 ng/ml (mean $\pm$ SEM) prior to administration of indoprofen and 182 ± 29 ng/ml before the lonazolac dose. After intake of either drug the TXB₂-levels decreased in all sera by more than 90 % of predose levels, but gradually returned towards higher levels as plasma was cleared of the drugs. In several samples the levels of TXB₂ were below the detection limit of the assay (5 pg/0.1 ml of serum

sample diluted 1:100) which usually corresponded to 2-3 % of the predose-levels. At 24 h after the 100 mg indoprofen dose 38 ± 6 % (mean $\pm$ SEM) of the predose-levels of TXB₂ were measured, whereas after the dose of lonazolac-Ca 200 mg 54 ± 4 % was recovered at 6 h and 94 ± 9 % at 24 h.

The TXB₂-levels were plotted against the concomitant plasma-concentrations of the respective drug, which revealed a concentration dependent inhibition of TXB₂-formation (Fig 3). For lonazolac the slope could be fitted to a straight line. When calculated for all data within limits of determination ($n = 39$), the value for r was -0.79 and the concentration of lonazolac at which TXB₂-formation was 50% of the predose level (IC₅₀) was 3.0×10^{-7} M. When excluding the values marked with arrows in Fig.3 the value for r was -0.89 , $n = 34$, $p < 0.001$, and IC₅₀ was 2.1×10^{-7} M. Pharmacological activity with the metabolite was also considered, and was calculated to be not more than 10% of that of lonazolac proper (Schrank, personal communication). TXB₂-formation was still concentration dependent, with a r -value for linear regression ($n = 34$) of -0.87 , when outlying points as described above were excluded. For indoprofen, only a small number of the total observations formed the slope. From linear regression analysis ($r = -0.92$, $n = 12$, $p < 0.001$) the IC₅₀ for indoprofen was extrapolated to 6.5×10^{-8} M, but by the eye IC₅₀ was estimated to fall at $1.4 - 1.5 \times 10^{-7}$ M.

For one male subject (subject nr 5) a considerably lower TXB₂-level was found prior to the dose of lonazolac than before indoprofen, but this could not be explained by any drug or food intake. At 24 hours after the lonazolac dose the TXB₂-level was 37% higher than prior to the dose.

Platelet aggregation

Platelet count in PRP (mean $\pm$ SEM, $n=7-8$) before and after single doses of indoprofen and lonazolac are presented in Table 1.

a) Lag-time to start of increase in light transmission.

From the lag-time measured as a response to various concentra-

tions of PRP, a value was calculated for the concentration of AA needed to give a lag-phase of 1 minute (AA_{1min}) in each batch of PRP. The lag-times tended to be shorter with higher platelet counts, but individual variations were great (Fig 4). In the presence of either drug the lag-time was prolonged or not identifiable within 5 minutes. In cases where the lag-phase could not be identified within 5 min, this was recorded as > 5 min, but for calculation of AA_{min} the value 5 min was used. However, with lonazolac but not with indoprofen, there was usually a gradual prolongation of the lag phase allowing a close titration of the AA_{min} with AA at concentrations between 0.5 and 1.5 mM. The AA_{1min} in relation to the concomitant drug concentrations in plasma are shown in Fig 5.

b) Rate of increase in light transmission during the first phase of aggregation

The maximum slope of the first phase of the aggregation curve (V_{max} ; scale units per min) is presented as the response to various concentrations of AA in presence of indoprofen or lonazolac (Fig 6) at 0, 2, 6 and 24 hours. The rate was somewhat higher before the indoprofen dose than before lonazolac, which reflects the higher platelet count in PRP. However, no difference could be found at 0 hour when PRP was stimulated with AA 1.0 and 1.5 mM, but the depressant effects of the drugs were most pronounced at these concentrations of AA. Indoprofen was more inhibitory than lonazolac at 2, 6 and 24 h ($p < 0.04$ or less at AA 1.5 mM). Indoprofen at 2 and 6 h was also more depressant than lonazolac at 6 and 24 h ($p < 0.05$ at AA 1.5 mM), respectively.

c) d) The change in light transmission over 5 minutes and the appearance of irreversible aggregation

Measured values for LT_{5min} and appearance of irreversible or reversible aggregation in response to various concentrations of AA are shown in Fig 7.

In absence of any drug a complete aggregatory response was not always elicited by AA-concentrations 0.3 and 0.5 mM. Appearance of

a complete response and irreversible aggregation was an all-or-none phenomenon, and a threshold concentration of AA could be identified for each PRP batch in absence of drug. With AA 2.0 mM, however, aggregation started always but often reversed spontaneously so that the LT after 5 min approached base-line levels. The AA concentration-response curve was thus bell-shaped. For subject nr 5, irreversible aggregation could not be elicited by any AA-concentration prior to lonazolac administration, but after 6 hours the aggregatory response reappeared (the reaction was not tested at 2 hours).

In presence of either drug, higher concentrations of AA were needed to elicit irreversible aggregation although in several PRP batches it did not occur at any concentration of AA tested. In some cases aggregation started normally and reached a high value for LT, but reversed towards the baseline. Both these phenomena were more common with indoprofen than with lonazolac, which is illustrated by the median values for LT in Fig 7. Like at 0 hours, the response was of the all-or-none type, and subsequently the range in LT_{5min} was wide.

The differences between the results in presence of either lonazolac or indoprofen at 2, 6 and 24 hours were tested by the Wilcoxon test. The differences were significant ($p < 0.02$) at 6 h (AA 1.5 mM), and at 24 h (AA 1.0 mM). P-values > 0.05 but < 0.10 were found for differences at 2 h (AA 1.5 and 2.0 mM), at 24 h (AA 0.5 and 1.5 mM) and for lonazolac at 6 h compared with indoprofen at 24 h (AA 1.5 mM).

Adverse effects

No major side effects occurred with either drug. One subject felt dizziness at 2-3 hours after indoprofen, another experienced a metal taste for two hours starting shortly after taking lonazolac, and a third subject reported headache in the afternoon of both trial days.

DISCUSSION

The present results show that the effects of indoprofen and lonazolac on platelet aggregation and on TXB_2 -formation in blood can be correlated to the drug concentration in plasma. The total concentration at which formation of TXB_2 was inhibited by 50 % (IC_{50}) can be estimated as $1.4 - 1.5 \times 10^{-7}$ M for indoprofen and as $2.1-3.0 \times 10^{-7}$ M for lonazolac. Like most other nonsteroidal antiinflammatory drugs both indoprofen and lonazolac are highly bound to plasma proteins, the figures given are 99% for indoprofen (Fuccella et al. 1973) and 97.3% for lonazolac (Rainer et al. 1981). If only the free fraction of the drug is active, the IC_{50} for indoprofen would be $1.4-1.5 \times 10^{-9}$ M and for lonazolac up to $6-9 \times 10^{-9}$ M. The somewhat higher unbound fraction of lonazolac will not completely compensate for its slightly lower potency when the total concentrations of the drugs are equal. Thus, the 10 times higher plasma-concentrations of indoprofen during the elimination phase cannot be motivated for equipotency on the cyclo-oxygenase in platelets. Instead, it was found that plasma-concentrations and effect on TXB_2 -inhibition of lonazolac 200 mg at 2 and 6 hours corresponded approximately to the concentration and effect of indoprofen 100 mg at 6 and 24 hours, respectively. It should be observed that both drugs have been used clinically at the dosage 200 mg x 3.

For both compounds the IC_{50} was close to the IC_{50} of 2.7×10^{-7} M previously found for indomethacin (Vinge 1985), but higher than that for fenflumizole (5.1×10^{-8} M; Vinge et al. 1984). Predose levels of TXB_2 were in the same range in all three studies, and correspond with the results reported by other groups using the same method (e.g. Alessandrini et al. 1985, Carter & Hanley 1985). As reported above, one of the subjects had a low TXB_2 -value prior to intake of lonazolac, accompanied by a deficient aggregatory response to AA, but no plausible explanation could be found. Interestingly, on one occasion previously this subject has shown a low predose TXB_2 -level, but has no signs of disease or bleeding disorder.

The aggregatory response to AA was inhibited by both indoprofen and lonazolac, but with different patterns. In drug-free PRP the duration of the lag phase is dependent on how much AA is added to the plasma (Lecompte et al. 1984, Vinge 1985). It is also dependent on the platelet count in PRP, but the variation between individuals seems to be more important than the number of platelets (Figure 4). In drug-free PRP it is usually possible to identify or interpolate the concentration of AA which gives a lag-phase of 1 minute. When indoprofen was present it was found that shape change did not always occur, or the lag phase was prolonged more than 5 minutes, whereas in the presence of lonazolac there was a gradual prolongation so that the lag phase could be determined by titration of the AA-concentrations. For indoprofen, the $AA_{1\text{ min}}$ had thus to be calculated from less secure values for T_{lag} . This may be due to a more pronounced effect of indoprofen on the subsequent first slope in the increase of light transmission (Fig 6), which may hide the lag phase. However, the $AA_{1\text{ min}}$ -values as a function of the concentration of each drug followed similar patterns for both drugs (Fig 5), and suggest an increase of $AA_{1\text{ min}}$ which is dependent on the drug concentration in plasma and which reaches a maximum of about 3 times the predose-values. Furthermore, the data for indoprofen suggest that the maximum effect is reached at plasma concentrations which correspond to those giving maximum inhibition of TXB_2 -formation. It has been demonstrated that the development of platelet shape change in response to AA or endoperoxide analogues can be inhibited by antagonists of the thromboxane receptor, such as 13-APA (Parise et al. 1984) and BM 13,177 (Patscheke & Stegmeier 1984). The change of shape of platelets in this assay may thus represent the activity of endoperoxides and thromboxanes formed by the platelets in response to exogenous AA.

The slope of the first phase of the light increase was found to be dependent on the AA-concentration used to induce aggregation (Fig 6). In drug-free PRP a maximum slope was found when aggregation was induced by 1.5 mM, an observation which corresponds with the bell-shaped aggregation response to AA observed previously

(Linder et al. 1979, Fratantoni & Poindexter 1981, Aharony et al. 1982). Therefore, higher concentrations of AA than 2.0 mM were not used for stimulation. The slope of the curve has commonly been used as a measure of aggregation induced by ADP or adrenaline (Thompson & Vickers 1985), and previously for AA-induced aggregation in the assessment of the antiplatelet effect of sulphinpyrazone (Maguire et al. 1981). A related approach was employed by Kirstein Pedersen et al. (1981) who evaluated sulphinpyrazone by measuring the time to reach 50 % of maximum alteration in light transmission (LT50%), which is dependent on both the lag phase and the slope of the first part of the light transmission increase. In the present study, indoprofen depressed the rate of aggregation more at 6 hours than did lonazolac at 2 hours, in spite of seemingly comparable inhibition of TXB₂ formation in clotting blood (>97.5% and >96.6%, respectively) although a difference cannot be entirely excluded. Unexpectedly, however, the effects of indoprofen on the rate of aggregation at 24 hours after drug intake were comparable to those of lonazolac at 2 hours (Figure 6), but suppression of TXB₂-formation by indoprofen was clearly lower (Figure 2).

Threshold proaggregatory concentrations and changes in the extent of light transmission are commonly used as measures of AA-induced platelet aggregation. In the present study the appearance of the second, final phase of aggregation followed an all-or-none pattern in drug-free PRP stimulated with AA 0.3-1.5 mM (Fig 7), which is compatible with a release phenomenon (Charo et al. 1977, Lecompte et al. 1984). However, when platelets were stimulated with AA 2.0 mM a decrease in LT, interpreted as desaggregation, occurred also after the second phase, which again illustrates the bell-shaped aggregatory response to AA (Linder et al. 1979, Fratantoni & Poindexter 1981, Aharony et al. 1982). The presence of any of the two drugs caused inhibition of the second phase, but inhibition by indoprofen was more pronounced. Like for the rate of aggregation, the result of indoprofen at 24 hours was most close to that of lonazolac at 2 hours (Fig 7), which again was not paralleled by the effect on endogenous TXB₂ (Fig 2). The long-

lasting effect of indoprofen is in accordance with the observation of inhibited platelet release reaction for more than 24 hours after an oral dose of indoprofen 50 mg (Pogliani et al. 1974).

For 7 of the subjects the platelet count in PRP was higher prior to the indoprofen dose than to the dose of lonazolac. The reason is not clear, but it is noticeable that all these subjects received the indoprofen dose first. A similar tendency was however not observed for the platelet count in whole blood. Higher platelet counts in PRP were reflected by a tendency towards greater values for the rate and the extent of aggregation ($V_{\max}$ and $LT_5 \text{ min}$). Similar observations have been made by others (Siess et al. 1981). In presence of the drugs the higher platelet count would then be expected to result in less pronounced effects by indoprofen. However, the effects of indoprofen were actually greatest at 2 and 6 hours, when the platelet counts were at their highest. Thus, the different patterns of inhibitory effects of the two drugs cannot be attributed to variations in platelet counts.

The results of the present study thus suggest that the anti-aggregatory effects of antiinflammatory drugs do not solely depend on their potency to inhibit thromboxane generation. The same conclusion has been made by Cockbill et al. (1979) from in vitro studies of platelet release with ASA, indomethacin and flurbiprofen. For inhibitors of thromboxane synthetase (Grimm et al, 1981, Jones et al. 1982) discrepancies between antiaggregatory effect and inhibition of thromboxane generation have been attributed to formation of endoperoxides or prostaglandins which themselves exert effects on platelets (Hamberg et al. 1974). For drugs acting on the cyclooxygenase, however, there may be differences in drug binding to the cyclooxygenase enzyme (Humes et al. 1981) or in their kinetic properties on the cellular level, like penetration into the target cells. It seems also possible that additional pharmacological actions may contribute to the antiaggregatory effects, possibly mediated via other arachidonic acid metabolites of the lipoxigenase-pathway (Aharony et al. 1982, McDonald-Gibson et al. 1984).

From the present study it is clear that doses of nonsteroid antiinflammatory drugs chosen for clinical use may vary widely in their impact on platelet function, in spite of small differences in potency on platelet cyclooxygenase. For lonazolac, like for indomethacin (Vinge 1985), the maximum inhibitory effect on TXB₂-formation is attained at a plasma concentration which roughly corresponds to "therapeutic concentrations" at steady-state with oral treatment, but for indoprofen "therapeutic concentrations" by far exceed the concentration needed to inhibit TXB₂ generation by >95%. Further studies are needed to investigate if a similar extent of cyclooxygenase suppression is attained in other tissues, and how this relates to the clinical effects. Further investigations are also needed before conclusions can be made regarding the clinical consequences on hemostasis of these two drugs.

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Table 1

Platelet counts in platelet rich plasma (means $\pm$ SEM) prior to and following oral doses of indoprofen and lonazolac.

	0h (=predose)	2 h	6 h	24 h
Indoprofen	353 $\pm$ 52	369 $\pm$ 33	380 $\pm$ 39	341 $\pm$ 40
Lonazolac	250 $\pm$ 37	285 $\pm$ 22	300 $\pm$ 35	247 $\pm$ 51

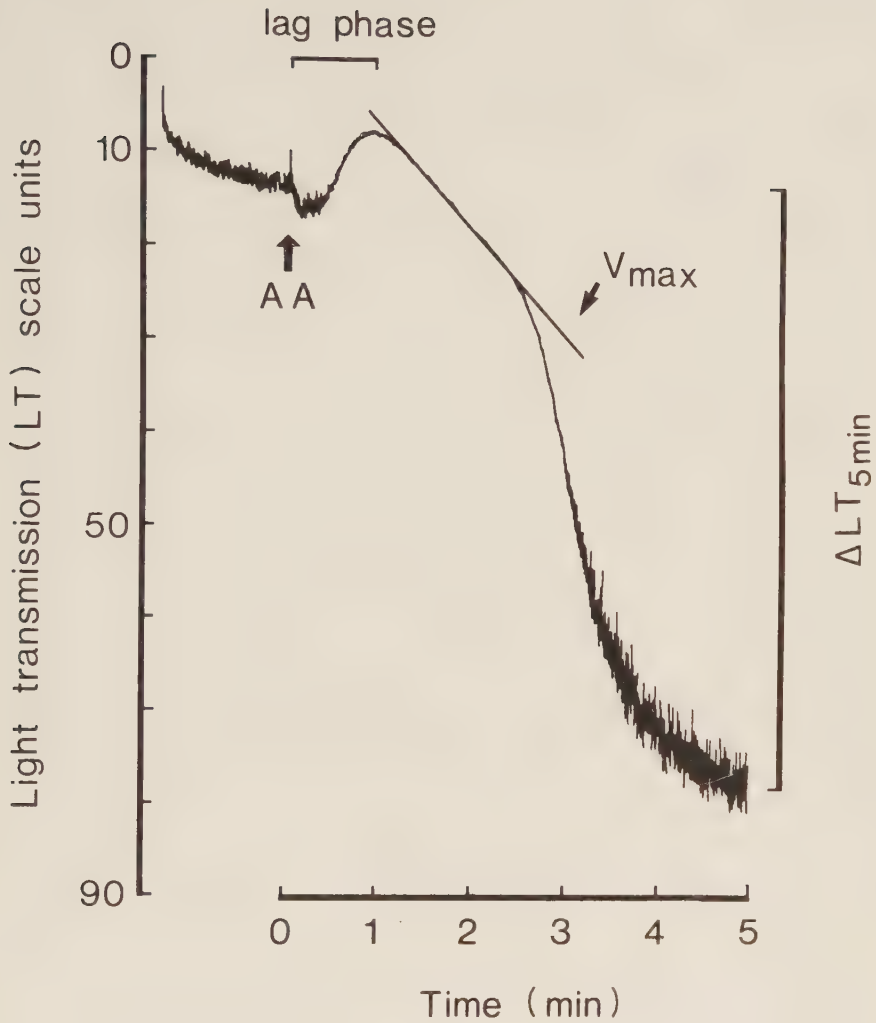


Figure 1:

Typical tracing of platelet aggregation in plasma induced by arachidonic acid (AA) 0.5mM added at arrow. The lag phase before start of aggregation, the maximum rate of aggregation (V_{max} ; represented by the slope of the tangent of the first part of the curve) and the change in light transmission over 5 minutes (ΔLT_{5min}) are demonstrated.

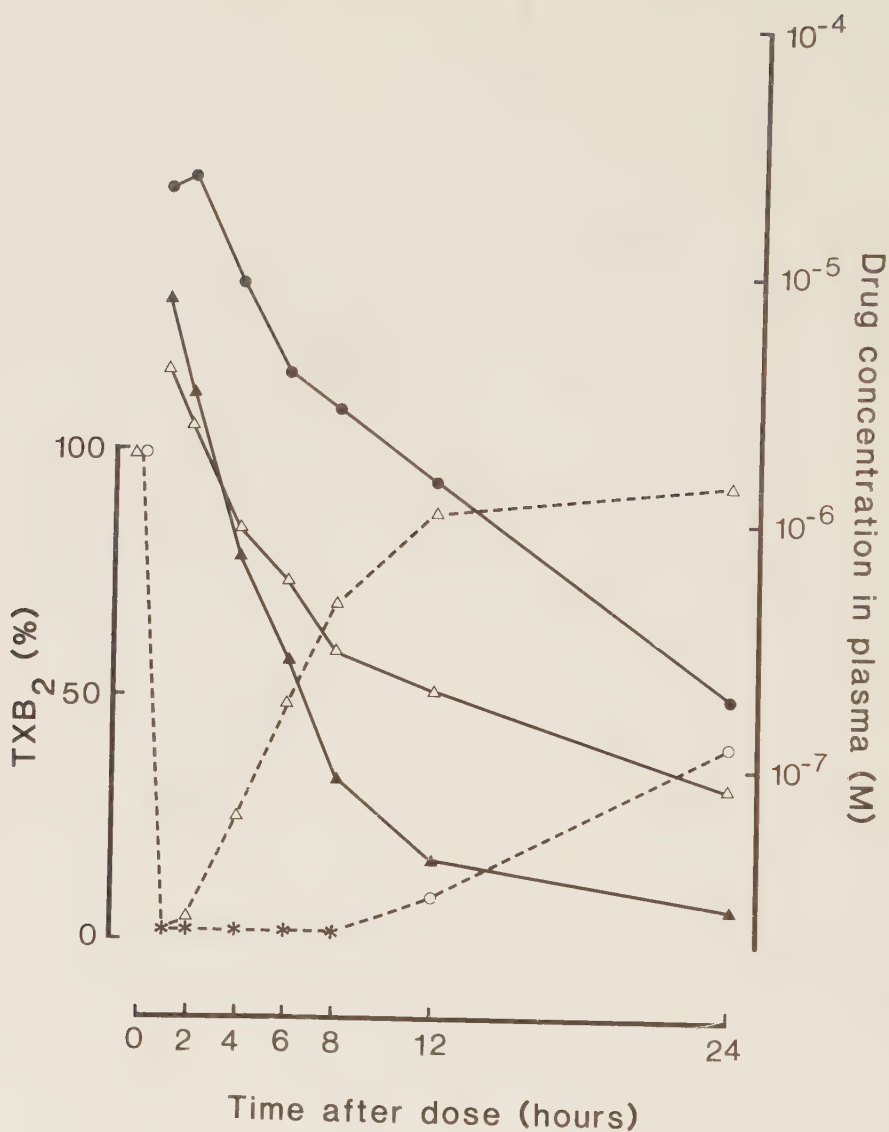
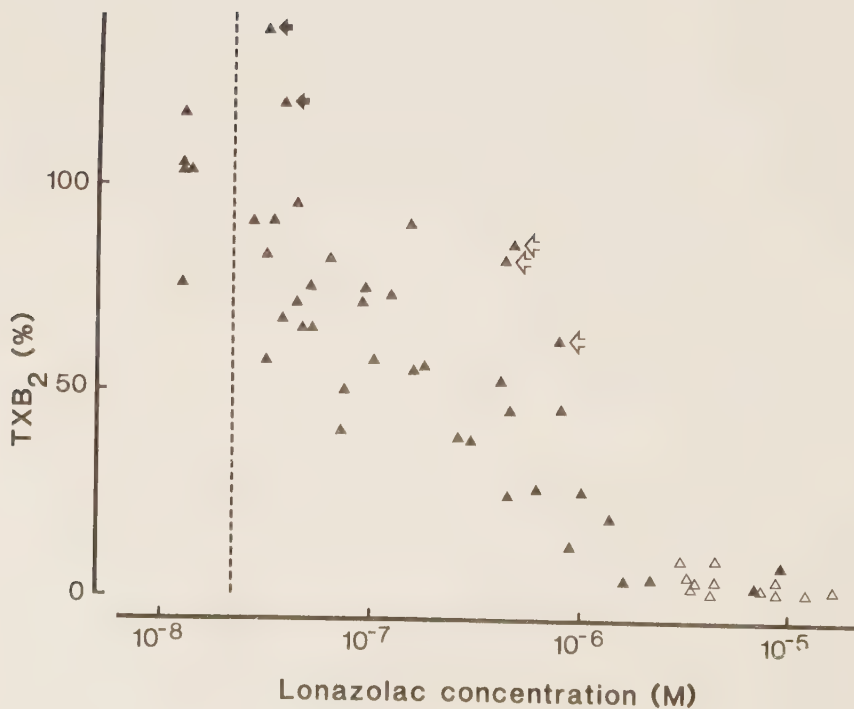
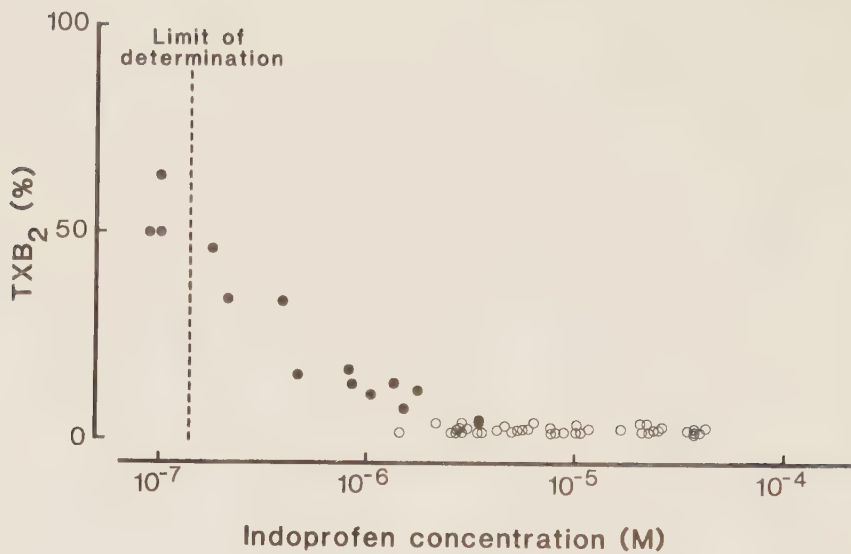


Figure 2:

Drug concentrations of indoprofen (●—●), lonazolac (▲—▲) and a metabolite of lonazolac (△—△) following single oral doses of indoprofen 100 mg and lonazolac-Ca 200 mg. Concomitant measurement of thromboxane B₂ formation in blood following the dose of indoprofen (0---0) and the dose of lonazolac-Ca (△---△) in % of pre-dose levels. Median levels for 8 volunteers. * symbolize median levels below the limit of determination. Note logarithmic scale for plasma concentrations, linear scale for TXB₂.

Figure 3 (next page):

TXB₂ formation in % of predose-levels as a function of plasma concentration of drug following single oral doses of indoprofen 100 mg (upper) and lonazolac-Ca 200 mg (lower). Open arrows point at results from subject no 2, and solid arrows point at data from subject no 5 (see text). Open circles or triangles mark the limit of determination of the TXB₂ assay when the levels were below this limit.



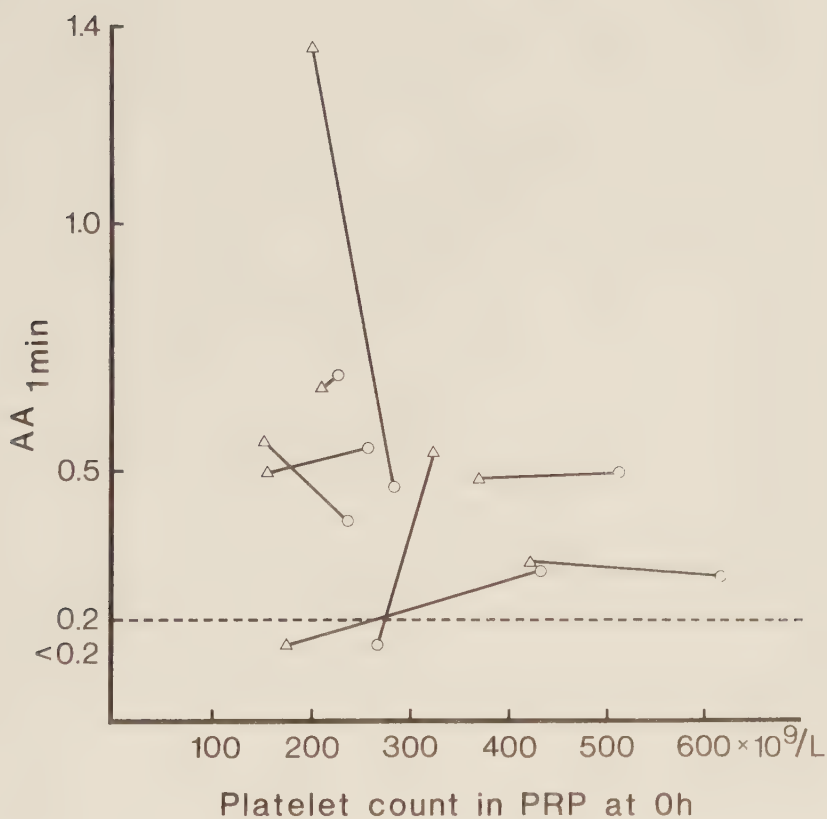
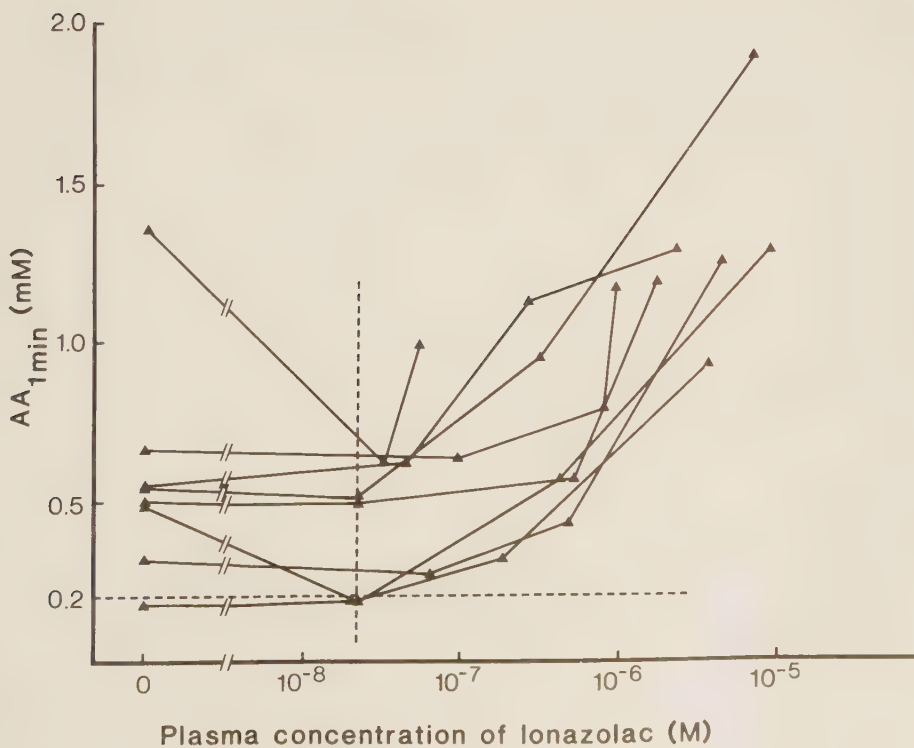
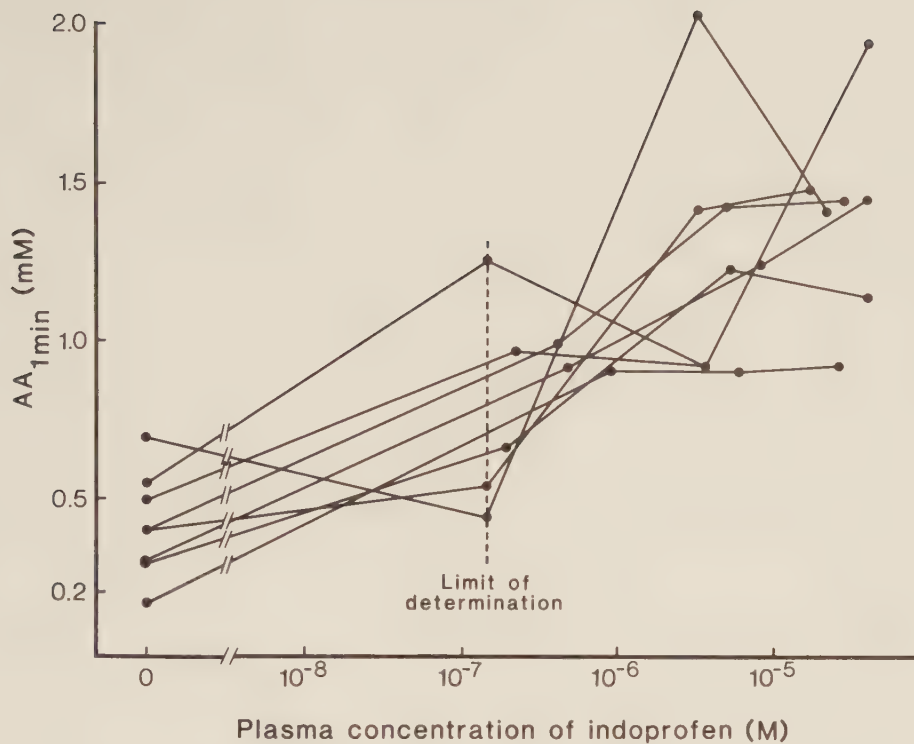


Figure 4:

Relation between value for $AA_{1 \text{ min}}$ in mM (see text) and platelet count in plasma prior to administration of indoprofen (O) and lonazolac-Ca (Δ). Results from the same subject are connected. A deficient aggregatory response was found for subject no 5, see text.

Figure 5 (next page):

Concentration of exogenous AA (mM) needed to give a lag phase of 1 minute ($AA_{1 \text{ min}}$) in platelet aggregation, related to concomitant plasma-concentration of indoprofen and lonazolac after single oral doses. Data from each individual are connected. A deficient response was found for subject no 5 immediately prior to administration of lonazolac.



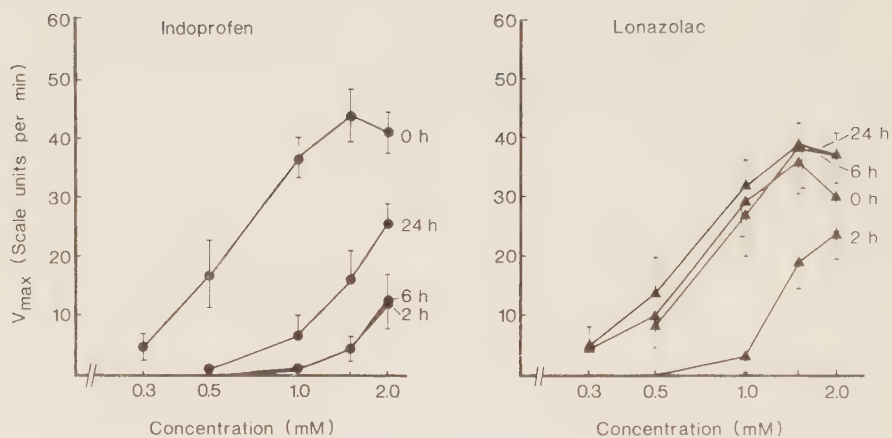


Figure 6:

Platelet aggregation rate (V_{max}) in response to various concentrations of AA prior to (=0h), and at 2, 6 and 24 hours after oral administration of indoprofen 100 mg and lonazolac-Ca 200 mg (means $\pm$ SEM, $n=7-8$).

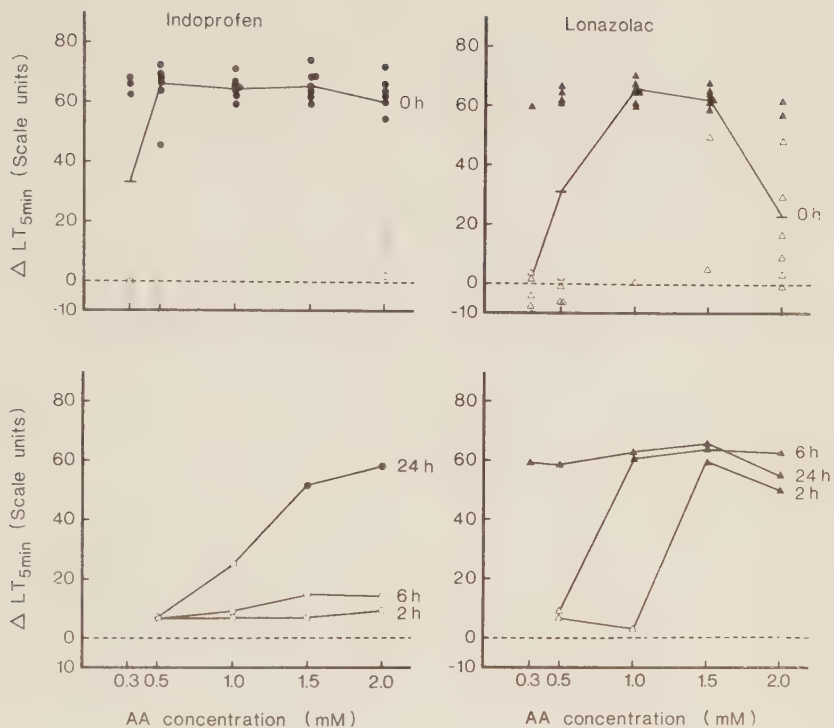


Figure 7:

Change in light transmission through platelet rich plasma over 5 minutes following platelet aggregation with various concentrations of AA prior to (=0h, upper), and at 2, 6, and 24 hours (lower) after oral administration of indoprofen 100 mg (left) and lonazolac-Ca 200 mg (right). In upper row, all individual data are presented; ● or ▲ represents irreversible aggregation, ○ or △ no reaction or reversibel aggregation, lines connect median values. In lower row only median values are shown; ● or ▲ symbolizes irreversible aggregation in at least half of the PRP tested.

